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raphic 114

RECENT ADVANCES

in the

STUDY *of* VENEREAL DISEASES

A Symposiu

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Held under the auspices of the Syphilis Study Section, Division of Research Grants and Fellowships, National Institute of Health U S Public Health Service in the auditorium of the U S Department of Commerce, Washington, D C, April 8 9 1948 Chairman, Syphilis Study Section

J E Moore

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ACKNOWLEDGEMENT

Publication of the proceedings of this symposium has been made possible through the cooperation of the Venereal Disease Division, U. S. Public Health Service and of the Venereal Disease Education Institute, Raleigh, North Carolina. This cooperation is acknowledged with sincere thanks.

FRANK W. REYNOLDS, M.D.
Executive Assistant, Syphilis Study Section
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FOREWORD

This symposium, the second to be held under the auspices of the Syphilis Study Section of the National Institute of Health, is a source of considerable gratification to all those who have been associated with the United States Public Health Service research grants program for the promotion of scientific investigations in medicine and allied fields To a considerable degree, the many excellent papers presented here represent achievements made possible by research grants to outstanding investigators working in the field of the venereal disease

The research grants program supports research which otherwise might not be conducted because of lack of availability of institutional or other funds This program does not take the place of support from foundations, private philanthropy, or private health organizations in medical and related fields of science The major objectives are (1) to expand research activities in universities and other institutions, (2) to stimulate the initiation of research in small colleges where previous research programs have been limited or non-existent, (3) to encourage investigators to undertake research in neglected areas, and (4) to provide *training for scientific personnel* *In carrying out* these objectives, the aim of the Public Health Service is to promote the highest quality of endeavors in both fundamental and applied research, without restrictive regulations There is no direction, control, supervision, regimentation or interference in the conduct of the research

The work that is being done throughout the country in the important field of the venereal diseases, and the accomplishments that are reported at this symposium, sustain the conviction that, as a result of scientific research, we may confidently anticipate profoundly beneficial effects upon the health of our nation

C J Van Slyke

C J VAN SLYKE, *Medical Director*
Chief, Division of Research Grants and
National Institute of Health
U S Public Health ᶜ
Federal Security

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A MESSAGE OF WELCOME

L A Scheele

(Surgeon General, U S Public Health Service)

It gives me a great deal of pleasure to be here today and to welcome to this symposium so many who are interested in the most recent advances in the study of the venereal diseases It is obvious from the program that we have in prospect two days of interesting and informative papers

The venereal diseases have constituted a major public health problem in this country for generations The Public Health Service is justifiably proud of its role in combatting the ravages of these diseases The scope and excellence of the program are tributes to the courage, resourcefulness and energy of one man—who, as much as any other, has made venereal disease control within these United States a foreseeable reality—Dr Thomas Parran Through his efforts and those of clinicians, administrators educators and investigators too numerous to mention, significant advances have been made in the struggle to stamp out the genito infectious diseases This struggle will continue until 'VD becomes a medical rarity in this country

I conceive research to be fundamental to the successful prosecution of the campaign We can by no means slight the purely medical and public health aspects of this problem, nor can we ignore its unique socio economic and moral implications, but we urgently need new methods of attack—and some of the most promising approaches require intelligent and intensive fundamental research for their development

To you, the outstanding investigators in this field, ever striving to interpret what actually exists—to you and to all those who by their attendance here may have their thinking catalysed in the interests of scientific progress, I extend a hearty welcome to this symposium You have my assurance that the campaign against venereal disease will continue, and that the research phases of this campaign receive my wholehearted endorsement and support,

I hope that we can meet like this from time to time, so that we all may benefit from the newer knowledge that continues to accrue as a result of scientific research in this important field

THE RATE OF DEVELOPMENT AND DEGREE OF ACQUIRED IMMUNITY IN EXPERIMENTAL RABBIT SYPHILIS

By
Harold J Magnuson and *Barbara J Rosenau**

This paper represents a final report on material presented in preliminary fashion at this meeting a year ago It is the first of a series utilizing a quantitative approach to immunity problems in experimental syphilis It is based on the concept that immunity may be relative and that the degrees of immunity may exist which will be adequate to combat minimal inoculums, but may be inadequate to overcome larger inoculums An animal's resistance may be measured by determining the size of inoculum required to produce infection The work reported this morning is concerned with the following variables (1) the duration of the original infection prior to treatment *vs* the degree of acquired immunity (2) the effect of penicillin *vs* mapharsen treatment in the development of this immunity

Time does not permit complete description of experimental details and only the most pertinent are included Throughout the present discussion the terms "original infection" or "immunizing infection" refer to the infection produced by the inoculation of *Treponema pallidum* into normal animals The terms "second infection" or "challenging infection" refer to the infection produced by the inoculation of *Treponema pallidum* into animals whose previous original infection had been cured by adequate treatment Uniform immunizing infections were produced by the inoculation of a known number of the Nichols strain of *T pallidum*, either into a single testis or intracutaneously at 6 sites on the rabbit's back The original infection was confirmed by darkfield examination and the immunizing infection was terminated at varying times after inoculation by curative treatment with either penicillin or mapharsen Penicillin as POB was given as 4 daily intramuscular injections to a total of 64,000 units/kg The mapharsen was given in 4 daily injections to a total of 6 mg /kg These doses represent respectively 8 times the CD_{9} of penicillin and 2 times the CD_{95} of mapharsen

The adequacy of this treatment and the distribution of rabbits treated in the present experiment are shown in Table I

* The University of North Carolina School of Public Health

TABLE I

The Distribution of Rabbits by the Type and Time of Treatment and the Manner of Inoculation

Numbers in table refer to total number of animals in experiment. Numbers in parentheses refer to number of animals in whom results are unknown because of adventitious deaths

Time of Treatment	Treated with Penicillin			Treated with Mapharsen			Total			Total
	Site of Inoculation		Unreinoculated Controls	Site of Inoculation		Unreinoculated Controls	Site of Inoculation		Unreinoculated Controls	
	Testis	Skin		Testis	Skin		Testis	Skin		
3 Weeks	20	7(2)		20	6		40	13(2)		53
6 Weeks	17(3)	3(1)	10	16	3	10(1)	33(3)	6(1)	20(1)	59
12 Weeks	19(1)	5(1)	5(2)	a 7(2) b 17(2)	3 3	8 7(1)	43(5)	11(1)	20(3)	74
24 Weeks	21(1)	7	10(1)	19(1)	8(1)	6	46(2)	15(1)	16(1)	77
Total	83(5)	22(4)	25(3)	79(5)	23(1)	31(2)	162(10)	45(5)	56(5)	263

a Total dosage = 6 mgs /kg
b Total dosage = 24 mgs /kg

It may be seen that the animals were treated at 3, 6, 12 or 24 weeks after the original infection and that of 263 animals entering the experiment 56 were allowed to go as unreinoculated treatment controls With but one exception, all of these animals were cured as judged by subsequent node transfer 4½ months after treatment This single exception occurred in animals treated with 6 mg /kg of mapharsen twelve weeks after inoculation While the possibility of technical error in this animal could not be excluded, it was felt that further animals treated at this time period should be given larger doses of mapharsen and these animals were given a total of 24 mg /kg The results of reinoculation in the two groups did not differ and the results are not distinguished in the tables that follow

Six weeks after treatment the animals were reinoculated with graded inoculums of *T pallidum* at the site of the original inoculation As controls on the emulsions used for these reinoculations, normal rabbits were inoculated with the same emulsion at six different sites over the back of the animal The results on these controls are summarized in Table II It may be seen that the mean incubation periods with these emulsions agree remarkably well with data previously reported and with but two exceptions the mean incubation periods for the controls of any one date fell within plus or minus one standard deviation of the average of the entire group These data indicate that the inoculum used for reinoculation was, on the whole, most uniform

Following reinoculation the animals were examined twice weekly for a three month period Table III shows the results of animals originally inoculated intratesticularly and reinoculated in the same manner Three types of results have been observed If a darkfield positive lesion developed at the site of reinoculation during the three month observation period, the animal was considered to have developed symptomatic reinfection When no local lesion developed, node transfers were performed in the usual manner three months after inoculation If the node transfer was positive, the parent animal was considered to have developed asymptomatic reinfection, and if the node transfer animals were negative, the parent animal was considered immune

The numbers in this table refer to results of individual rabbits inoculated with the indicated number of spirochetes It is apparent that as the duration of the original infection increases, progressively larger numbers of spirochetes are required to

produce a given response on reinoculation The immunity has been the same in the mapharsen and penicillin treated animals except in the group treated at twelve weeks The reason for this difference is not apparent As a measure of the increase in resistance we have calculated two end points for the various time periods The first is the symptomatic 50% infectious dose

TABLE II

Controls in Inocu ums Used for Re noculat on

Rabb ts were noculated ntracutaneo 1 at mult p e s tes w th 0 2cc of each em ul on N ambers n the table refer to the avera e ncuba o period of the s tes on control an mals of a given date

Date	Number Animal	Number of T pall dum n Emul on					
		10^6	10^5	10^4	10^3	10^2	10
3/11/46	2	13	7	29	46	50	
4/8/46	2	8	16	12 5	20 5	34 5	28
5/13/46	3	18	35	26	22	39	39
9/5/46	3	13	16	21	26	25	30
8/15/46	3	11	15	18	22	28	28
12/16/46	3	14	14	17	19	23	26
12/30/46	3	17	19	21	15	22	19
2/5/47	3	16	18	23	28	30	
3/21/47	3	9	13	14	23	26	
5/23/47	3	10	10	15	23	18	38
6/27/47	3	12	12	14	12	18	23
7/11/47	3	10	13	17	21	22	29
7/25/47	3	9	12	15	23	35	32
8/18/47	3	20	35	44	53	53	
9/19/47	2	10	18	24	27	36	
10/29/47	2	16	16	22	24	30	
Mean ± standard deviation		13 0± 4 9	16 3± 8 5	20 7± 8 8	24 3± 10 4	29 4± 12 3	30 8± 13 1
Mean ± standard deviation *		14 3± 4 6	17 1± 9 0	26 5± 11 4	26 7± 8 0	31 7± 10 6	34 9± 9 1

* Previous data of Magnuson Eagle & Fleischman

TABLE III

Development of Acquired Immunity in experimental Syphilis

Summary of Intratesticular Inoculations

Time of Rx	Inoculum	Treatment: Penicillin S	A	I	Treatment: Mapharsen S	A	I	Total S	A	I	Symptomatic ID_{50}	Asymptomatic ID_{50}
Three weeks	$2x10^4$	1			1			2			4 7x10	5
	$2x10^3$	4			4			8				
	$2x10^2$	3	1		2	3		5	4			
	2x10	2	3		2	3		4	6			
	2	1		4	1		4	2		8		
Six weeks	$2x10^3$	1	2		2	2		3	4		1 $5x10^3$	4 3x10
	$2x10^2$			3		3	1		3	4		
	2x10	2	1	1		3	2	2	4	3		
	2			4			3			7		
Twelve weeks	10^7		2	2		2			4	2	$>10^7$	$5x10^3 \pm$
	$2x10^5$		2	2		3			5	2		
	$2x10^4$		2	2		4			6	2		
	$2x10^3$		1	2		2			3	2		
	$2x10^2$		2	1			3		2	4		
Twenty-four weeks	10^8		6	1					6	1	$>10^8$	$2x10^5$
	$2x10^6$	1	2	2		3	2	1	5	4		
	$2x10^5$		2	3		1	3		3	6		
	$2x10^4$		2	7		1	3		3	10		
	$2x10^3$		2	3		2	3		4	6		

"S" = Symptomatic reinfection
"A" = Asymptomatic reinfectio
"I" = Immune

Rabbits were inoculated in a single testis with 10^6 *T pallidum* Treatment with curative doses of penicillin or mapharsen was given at time indicated, and reinoculations performed 6 weeks after treatment The site of the original inoculation and of reinoculation was the same The development of darkfield positive lesions at the site of reinoculation during a three months observation period was considered symptomatic reinfection When no local lesion developed, node transfers were performed three months after inoculation If the node transfer was positive, the parent animal was considered to have developed asymptomatic reinfection, and if negative, the animal was considered immune

TABLE IV

Development of Acquired Immunity in Experimental Syphilis Summary of Intracutaneous Inoculation

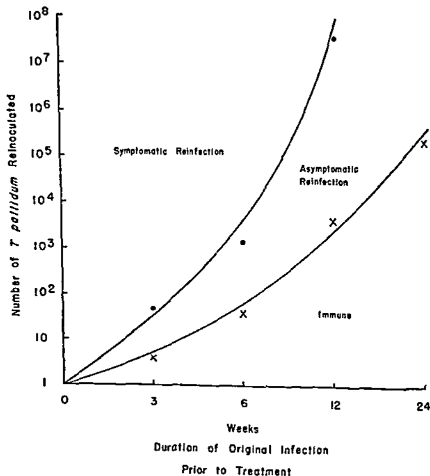
Rabbits were originally inoculated intracutaneously at six sites with serial 10-fold dilutions of a calibrated testicular emulsion to a total of 2 2 x 10^5 organisms, and were treated at the time indicated with either penicillin (4 daily injections of POB to a total of 64 000 U kg) or with mapharsen (4 daily injections to a total of 6 0 mg/kg) Intracutaneous reinoculation six weeks after treatment was similar to the original inoculation Node transfers were made three months after reinoculation on animals failing to develop symptomatic reinfection

	No of T P Reinoculated	*Time of Treatment*							
		3 weeks		6 weeks		12 weeks		24 weeks	
		+	—	+	—	+	—	+	—
Results of Reinoculation by sites	10^7		—	—	—	—		0	3
	2 x 10^6			—		—		0	3
	2 x 10^5	11	0	3	2	1	9	0	14
	2 x 10^4	11	0	3	2	2	8	0	14
	2 x 10^3	11	0	3	2	1	9	0	14
	2 x 10^2	11	0	3	2	1	9	0	14
	2 x 10	8	3	1	4	1	9	0	11
	2	4	7	0	5	1	9	0	11
	Symptomatic ID$_{50}$	5		2 x 10^3		2 x 10^5		10^7	
		No	%	No	%	No	%	No	%
Results of Reinoculation by Animals	Symptomatic	11	100	4	80	2	20	0	0
	Asymptomatic	0	0	1	20	6	60	8	57
	Immune	0	0	0	0	2	20	6	43

which represents the number of spirochetes required to produce symptomatic infection in 50% of the animals, the remainder of the animals being either asymptomatically reinfected or immune The second end point is the asymptomatic 50% infectious dose, which is the number of spirochetes required to produce either asymptomatic reinfection or symptomatic reinfection in 50% of these animals, the remainder of the animals being im-

Fig 1

Results of Intratesticular Inoculation



mune The increase in these two end points is evident from the table

The data are better summarized in Fig. 1 in which the two 50% infectious doses are shown graphically It is pertinent at this point to refer to our earlier published work in which it was shown that under carefully controlled conditions the infectious dose for rabbits on intratesticular inoculation was one spirochete In this same paper it was shown that in these same animals asymptomatic infection was extremely rare It follows, therefore, that in the normal animals the symptomatic ID_{50} and asymptomatic ID_{50} as defined above is one spirochete Starting from this point at 0 time we can see the progressive increase in the 50% infectious doses as the duration of the original infection is increased These two lines divide the graph into three broad areas Points within these respective areas represent combinations of challenging inoculums and durations of immunizing infections that may be expected to yield symptomatic reinfection, asymptomatic reinfections, and immunity It should be emphasized that these curves represent a summary of all of the data and that particularly in the later stages of the infection when the end points are not sharp, it is not possible to predict a certain outcome for a given rabbit with a given inoculum.

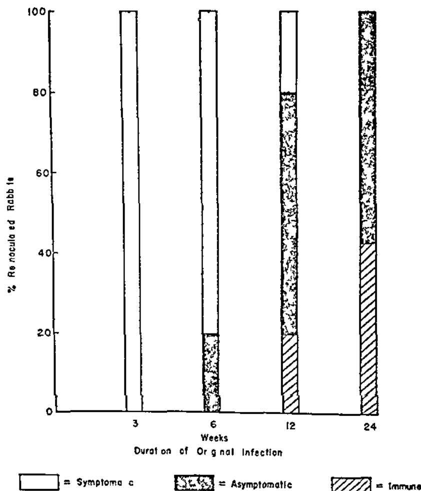
Table IV shows the results of intracutaneous inoculations in which the size of the challenging inoculum was kept constant The use of graded multiple intracutaneous inoculations permits rapid determination of the symptomatic 50% infectious dose, but complete immunity and asymptomatic reinfection become apparent only when the animal's resistance is sufficient to overcome the largest number of spirochetes The graphic presentation in this table shows that with a constant inoculum the change in immunity with the passage of time is reflected in a decrease in the number of symptomatic reinfections, an increasing proportion of asymptomatic reinfections, and finally some true immunity These results confirm those of the earlier workers using qualitative technics

The dangers inherent in transferring animal data to human diseases are recognized It is reasonable to assume, however, that some number of *Treponema pallidum* represents a minimal infectious inoculum in man If acquired immunity exists in man and develops in a progressive manner, there will be a progressive increase in the number of *Treponema pallidum* required to produce either symptomatic or asymptomatic reinfec-

tion Since the size of inoculums in humans are unknown and since these inoculums probably vary over wide limits, the clinical expression of developing acquired immunity will be limited to changes in the ratios of symptomatic reinfection asymptomatic reinfection, and immunity

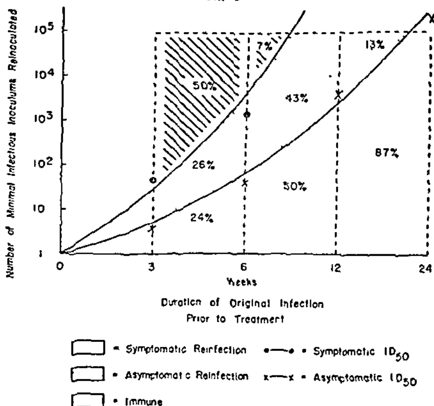
While there now exist no clinical means to differentiate relapse from reinfection, it is perhaps instructive to consider the effect of increasing immunity in relation to apparent cure rates

Fig 2
Intracutaneous Inoculation of 2.22×10^5 T pallidum



in various stages of syphilis. For sake of illustration, let us assume that animals treated within the three to six weeks period following inoculation are comparable to humans treated in the primary stage, animals in the six to twelve weeks periods to humans in the secondary stage, and from twelve to twenty-four weeks to the early latent stage. Further, let us assume that an infinite number of patients are reinoculated with from 1 to 100,000 minimal infectious inoculums of *T. pallidum.* In Fig. 3 these limits have been superimposed on original symtomatic and asymptomatic ID_{50} curves of Fig. 1. The relative areas between these limits and their respective curves are shown. Thus, under the conditions assumed, patients originally treated in the primary stage might be expected on reinoculation to develop symptomatic reinfection 50% of the time, asymptomatic reinfection 26% of the time, and escape infection 24% of the time. Similarly, with patients treated in the secondary stage

Fig 3



7% might be expected as symptomatic reinfection, 43% asymptomatic reinfection, and 50% no infection Of patients treated in the early latent stage none would develop symptomatic reinfection 13% asymptomatic reinfection, and 87% no infection These percentages are given only to indicate trends in the type of clinical response following reinoculation If the inoculums to whim humans are exposed vary in the pattern of a normal curve rather uniformly as was assumed, then the responses to medium size inoculums will be increased Regardless of absolute percentages, the chief importance of the argument is that patients treated in the primary stage are most apt to have symptomatic reinfection Those treated in the secondary stages may be expected to have more asymptomatic reinfections, which will be clinically manifest (if at all) by apparent serological relapse Neither symptomatic nor asymptomatic reinfection will be of importance in those treated later in the disease

Time does not permit a complete discussion of the serologic results in these animals, but I shall mention a few findings which may have clinical interest In the first place, there has been no relationship between the degree of immunity and the serologic titer in these animals In the second place, asymptomatic reinfection occurring in animals whose first infection has been terminated early has usually been associated with a serologic relapse, but where reinfection has occurred in animals treated later there has not been such a relapse Third, experimental reinfection resulting in clinical symptomatic reinfection may actually be preceded by an increase in serologic titer The apparent serologic relapse antedates the clinical lesion

THE RATE OF MULTIPLICATION OF TREPONEMA PALLIDUM IN NORMAL AND IMMUNE RABBITS

By

Mary C Cumberland and *Thomas B Turner**

Little is known concerning the succession of events which occur soon after the introduction of virulent *Treponema pallidum* into a susceptible or a resistant host It is known that with the experimental methods commonly employed, treponemes rapidly migrate or are carried beyond the site of inoculation to invade the regional lymph nodes and the blood stream It is also known that after about 5 days minimal histological changes can be recognized in susceptible animals, although microscopically detectable lesions do not often occur before the 10th to 14th day

The present study is an attempt to determine what happens to spirochetes during the first few days following introduction into a susceptible or a resistant host

Groups of rabbits having testes of approximately the same size were inoculated with an emulsion prepared from testicular syphilomas, Nichols strain, and diluted so that the desired number of spirochetes was contained in a volume of 0 2 cc Care was taken to place the inoculum into the middle of the testis At various intervals after inoculation testes were removed and cut into six equal transverse sections A loopful of serum saline was pressed into the surface of each segment and the tissue-rich fluid collected on a coverslip Using a darkfield microscope 50 high power fields of each preparation were examined, and the total number of spirochetes counted in all six preparations was taken as an index of the spirochete population of the whole testes

Immune rabbits were obtained by intracutaneous inoculation of the back with the Nichols strain of *T pallidum* Seventy to eighty days after infection the animals were treated with a curative dose of penicillin in order to eliminate any spirochetes present in the testis prior to intratesticular introduction of the challenge inoculum

Figure 1 shows the median counts obtained in testes of normal rabbits on various days according to the number of spirochetes inoculated By comparing the number inoculated into the

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* [illegible] The Johns Hopkins University,
[illegible]more Md
[illegible]y grants from the Division of
[illegible]al Institute of Health, U S
[illegible] Health Division of The Rocke

testis with the number counted when the testis is removed immediately after inoculation the ratio of spirochetes counted to spirochetes present in the testis is about 1 to 125,000

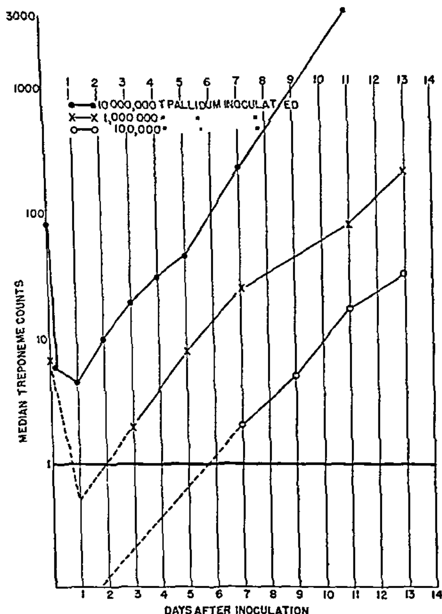


FIG. I RESULTS OF TREPONEME COUNTS ON TESTES OF NORMAL RABBITS AT VARIOUS PERIODS FOLLOWING INOCULATION WITH GRADED NUMBERS OF T PALLIDUM

Within 2 hours following inoculation with 10 million spirochetes, there has occurred about a 90% reduction in the number of organisms present in the testis Apparently the majority of the treponemes have migrated or been carried from the testis by either vascular or lymphatic routes Following the rapid initial loss logarithmic multiplication of spirochetes takes place at a constant rate regardless of whether the initial inoculum consisted of 10 million, 1 million or 100,000 organisms An average of 108 hours or 4½ days is required for a tenfold increase in the number of treponemes This is equivalent to an average of 33 hours necessary for a single division, assuming that each spirochete divides into two spirochetes

The following table (Table I) gives the results of intratesticular inoculation of 10 million treponemes into normal and immune rabbits About 90% of the organisms inoculated into both the normal and immune rabbit testis have disappeared within 2 hours Spirochete counts made at 24 hours after inoculation show no differences between normal and immune testes but by 5 days post-inoculation the number of treponemes found in the testes of immune animals is definitely lower than in normal rabbits The 7 and 11 day counts show very striking differences between normal and immune animals Spirochetes in the normal testes have become exceedingly numerous whereas in the majority of the immune testes none or only a few organisms are visible

TABLE I

Results of Treponeme Counts on Testes of Normal and Immune Rabbits at Various Periods Following Inoculation with 10 M on *T pallidum*

Time After Inoculation	Median		Range of Counts	
	Normal	Immune	Normal	Immune
Immediate	81	77	30 202	52 109
2 hours	6	5	0-13	0-9
1 day	4 5	1	0-45	0-29
3 days	19 5	6 5	0-51	0-95
7 days	232	1	75-369	0 201
11 days	2998	0	1143-4924	0-1

The last column of the table indicates that in certain individuals among the immune animals examined at 3 or 7 days, there is a multiplication occurring at approximately the same rate as in normal animals Since in the group of so-called immune rabbits, there was undoubtedly great variation in the actual degree of immunity in individual animals, such high counts may have occurred in animals which had little or no acquired resistance Perhaps eventually a clinical orchitis would

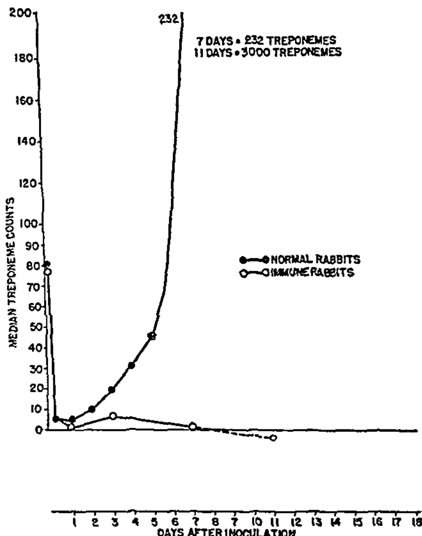


FIG 2 RESULTS OF TREPONEME COUNTS ON TESTES OF NORMAL AND IMMUNE RABBITS AT VARIOUS PERIODS FOLLOWING INOCULATION WITH 10 MILLION T PALLIDUM.

have occurred in these animals On the other hand, such high counts may simply indicate that the degree of immunity was not sufficient to prevent two or three cycles of division, following which the spirochetes would have ceased to increase in numbers, and might have slowly died off

Figure 2 shows the results plotted in graphic form The growth curve of treponemes inoculated into normal animals is typical of growth curves for other types of micro organisms Spirochetes introduced into immune animals have not significantly increased in number after eleven days and, in fact, are probably decreasing

The rate of multiplication of *Treponema pallidum* in the normal rabbit testis as computed by the method described is approximately 33 hours per single division This figure may be compared with that obtained by Magnuson, Eagle and Fleischman[1] who calculated a division time of 30 hours from data on the incubation period of back lesions following intracutaneous inoculation of graded numbers of treponemes It should be emphasized that all such figures indicate only the rate at which the number of spirochetes increases at the site of inoculation and not necessarily the actual time required for 1 spirochete to divide into 2 Since it has been shown that about 90% of the original inoculum has left the testis within two hours, it is possible that there is a continuous outflow and, perhaps, inflow of spirochetes in the testis following the initial rapid loss Unfortunately, virtually nothing is known concerning the rate at which spirochetes leave the testis It is possible that, following the early initial migration, the rate of outflow becomes stabilized at a rather low level It is assumed, also, that some spirochetes re-enter the testis from the blood, but the numbers are probably minimal

In spite of the fact that the so called immune animals were not solidly immune, in general spirochetes did not multiply in their testes Apparently there is no mechanism whereby the immune animal can kill or immobilize the organisms immediately after their entry into the body, but they are prevented from dividing, so that eventually they die

These experiments present further evidence that immunity to experimental syphilis is not localized but is present in tissues far from the site of the initial chancre In view of other studies

carried on in this laboratory, it is probable that humoral factors play a significant role in this phenomenon

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THE ANTIBODY RESPONSE IN RABBITS TO KILLED SUSPENSIONS OF PATHOGENIC *T PALLIDUM**

By
Harry Eagle and *Ralph Fleischman*†

Although reinfection with syphilis is not uncommon, it is usually observed in patients who have been treated early in the disease, and is observed only rarely in patients treated after the secondary lesions have spontaneously resolved[1] In syphilitic rabbits also, a solid immunity develops after a period of approximately 3 months[2] If such animals are cured by appropriate treatment and then reinoculated, even several million organisms usually fail to produce a second infection

The mechanism of this immunity is not clear It bears no obvious relationship to the presence of serum agglutinins or lysins, and opsonins have not been demonstrated Syphilis does regularly cause the production of an antibody ("reagin") reactive with a ubiquitous lipoidal component of normal mammalian tissue It is not yet clear, however, whether this reagin is actually a specific antibody to *T pallidum* which happens to cross-react with a serologically related component of mammalian tissue[3], or whether, as suggested by Sachs, Klopstock, and Weil[4], syphilis causes the breakdown of host tissue and the protein to form a complete antigen In either case, the presence of so called Wassermann or flocculation reagin is apparently not

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* This manuscript represents excerpts from a more detailed paper which will appear in the Journal of Experimental Medicine

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the basis of the observed resistance to reinfection. In both rabbits and man, the titer of this antibody is highest in the early stages of the disease, long before there is a significant degree of immunity, and the titer may have dropped to negligible levels at a time when the immunity is maximal

The only experimental evidence yet adduced for the presence in the serum of syphilitic animals or men of antibodies directed specifically against the causative organism in the demonstrations by Tani and his coworkers[5,6] and by Turner[7] that when such serum is incubated with suspensions of *T pallidum* and the mixtures injected into rabbits, the production of syphilitic lesions is then either delayed or prevented In the latter instance, it is not yet known whether the admixture with serum actually prevents infection, or merely suppresses the appearance of the primary lesion, and makes the infection asymptomatic

The present experiments were undertaken to determine the antibody response in rabbits to killed suspensions of pathogenic *T pallidum*, administered in saline suspension, or incorporated in oil-in water emulsions as described by Freund[8] As will be here shown, the injection of even large numbers of the organisms, up to a maximum of 38 billion injected over a period of 13 weeks, did not produce demonstrable resistance to infection, since as few as ten organisms then sufficed to cause infection The serum of such "immunized" animals had no demonstrable direct effect on the organisms *in vitro*

The injection into rabbits of killed *T pallidum* did, however, regularly cause the production in high titer of antibodies similar to those which develop in the course of syphilitic infection, the serum giving positive complement fixation (Wassermann) and flocculation tests with lipoidal extracts of normal tissue The serum dilution titers reached a maximum level within 2 to 3 weeks Thereafter, and despite continuing injection, the titers decreased progressively

The present experiments therefore throw no light on the mechanism of immunity in syphilis They do, however, constitute strong evidence that, as originally postulated by Wassermann, so called reagin may be simply an antibody to *T pallidum* Its anomalous reactivity with alcoholic extracts of normal mammalian tissue is probably to be attributed to the presence in such extracts of a lipid substance serologically related to one or more of the antigenic components of the organism (*cf* [3]) Methods of preparing treponematal suspensions from rabbit chancres

have not yet yielded suspensions sufficiently concentrated and sufficiently free from tissue components to warrant either their chemical fractionation or their use in absorption experiments

EXPEPIMENTAL

There will be here described one of 5 experiments in which saline extracts of rabbit testicular chancres were used as antigen

Testes were removed at the height of the inflammatory reaction 10 to 14 days after their inoculation finely minced with scissors and ground in a mortar and pestle with 0 85 per cent NaCl (approximately 5 cc per testis) The mixture was lightly centrifuged to remove the tissue particles and the sediment reextracted with a second portion of salt solution The combined supernatant fluids were centrifuged in a conical head (International Centrifuge Co No 923) for 1 hour at the end of which time from 75 to 90 per cent of the organisms had been sedimented as a whitish pellicle The number of organisms in the sediment was estimated by counts on the suspension before and after centrifugation The supernatant fluid was drained the sediment was resuspended in salt solution to give a final concentration of 1 billion organisms per cc and the suspension frozen at —25°C Over a period of 2 months nine such suspensions totalling 140 cc were prepared from 192 testicular chancres Some of these suspensions were killed before freezing by the addition of merthiolate to a 1 1000 concentration and warming to 37°C for 1 hour The others were kept frozen until they were no longer infectious No difference was noted between the two types of suspension and they are not distinguished in the text

In the first experiment with this material thirteen rabbits were injected three times weekly to a total of sixteen injections in a period of 46 days After the seventh injection there was a rest period of 12 days before injections were resumed The dosage per injection averaged 0 5 cc, or 500 million organisms and the total number of treponemata injected during the immunization period was 7 billion

As is indicated in Table I, every animal injected developed Wassermann and flocculation antibodies within a period of 16 days, the flocculation titers at this time varying from 1 16 to 1 64 No significant increase in this titer was observed on prolonged immunization Instead in most of the animals there was an indication that the serologic titer had reached a maximum in the first 2 weeks and thereafter decreased Three days after the last injection, a popliteal node was removed from the surviving animals, emulsified in 25 per cent serum, and injected into a normal rabbit to demonstrate that the suspensions used for immunization had actually been killed, and that the immunized animal had not been infected None of the nodes proved

the basis of the observed resistance to reinfection. In both rabbits and man, the titer of this antibody is highest in the early stages of the disease, long before there is a significant degree of immunity, and the titer may have dropped to negligible levels at a time when the immunity is maximal.

The only experimental evidence yet adduced for the presence in the serum of syphilitic animals or men of antibodies directed specifically against the causative organism in the demonstrations by Tani and his coworkers[5,6] and by Turner[7] that when such serum is incubated with suspensions of *T. pallidum* and the mixtures injected into rabbits, the production of syphilitic lesions is then either delayed or prevented. In the latter instance, it is not yet known whether the admixture with serum actually prevents infection, or merely suppresses the appearance of the primary lesion, and makes the infection asymptomatic.

The present experiments were undertaken to determine the antibody response in rabbits to killed suspensions of pathogenic *T. pallidum*, administered in saline suspension, or incorporated in oil-in-water emulsions as described by Freund[8]. As will be here shown, the injection of even large numbers of the organisms, up to a maximum of 38 billion injected over a period of 13 weeks, did not produce demonstrable resistance to infection, since as few as ten organisms then sufficed to cause infection. The serum of such "immunized" animals had no demonstrable direct effect on the organisms *in vitro*.

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The present experiments therefore throw no light on the mechanism of immunity in syphilis. They do, however, constitute strong evidence that, as originally postulated by Wassermann, so-called reagin may be simply an antibody to *T. pallidum*. Its anomalous reactivity with alcoholic extracts of normal mammalian tissue is probably to be attributed to the presence in such extracts of a lipid substance serologically related to one or more of the antigenic components of the organism (*cf.*[3]). Methods of preparing treponematal suspensions from rabbit chancres

TABLE II

Incubation Period on Inoculation of Rabbits with living T pallidum 4 Days after the Last Immunizing Injection

Rabbits of Table I

No of organisms inoculated	Rabbit No (cf Table I)	Incubation period in time after	
		Intradermal inoculation	Intratesticular inoculation
		days	*days*
100 000	67 75	17	67
	67 70	13	42
	Normal controls simultaneously inoculated	9 13 13	21 28 35
1 000	66-62	21	28
	67 28	28	77
	67-61	21	35
	Controls	21 21 21	28 28 28
10	66-87	28	-*
	67 79	28	*
	67 80	28	42
	Controls	28 35	42 42 92

* No obvious testicular involvement for 3 months after inoculation and testis dark field negative at that time

large inocula[11] Accordingly in a second experiment with sedimented organisms an average of 1 billion killed organisms was injected three times weekly for a period of 4 months and the total number of organisms injected was 38 5 billion

Maximum titers were again obtained within 2 weeks Thereafter, however, the titers fell continuously, despite continuing injections at the same high level Thus, the flocculation titer at the end of 2, 4 6 9, and 18 weeks averaged 28, 14, 6 5, 4 5, and 4 5, respectively When 100 or 10 organisms were injected into two animals intradermally at the end of the immunization period, a syphilitic lesion developed within a normal incubation period As in the previous experiment, however, there was a suggestion of resistance in animals inoculated intratesticularly Although two rabbits inoculated with 100 organisms both developed a chancre, the incubation period was prolonged in one of the two Of two other rabbits inoculated with 10 organisms, one failed to develop a lesion A popliteal lymph node transferred to a normal

animal 110 days after the original inoculation proved infectious, indicating that the animal had undergone an asymptomatic infection Despite these suggestions of an altered response, it is nevertheless clear that no significant degree of immunity had developed in these animals under the impact of relatively large numbers of dead organisms

The serum from one of these rabbits, obtained midway in the immunization period 63 days after the first injection, had only a questionable direct effect on pathogenic *T pallidum* The serum, either whole or diluted 1 10, was incubated with an equal volume of a chancre emulsion containing 10^8, 10^6, 10^4, 10^2 organisms per cc, and 0 2 cc of each mixture, representing inocula 10^7, 10^5, 10^3, or 10 treponemata, was then injected into normal rabbits intradermally or intratesticularly The three larger inocula caused the appearance of typical darkfield positive chancres within a normal incubation period However, the suspension containing the smallest number of organisms proved non infectious on intratesticular inoculation into two rabbits, this despite the fact that one to two treponemata have been shown to be regularly infectious for rabbits on intratesticular inoculation[10] When the same suspension was similarly incubated with normal serum and inoculated, typical chancres developed in 28 to 35 days The significance of this observation is nevertheless open to question When 1 10 serum was used instead of whole serum, the "immune" and normal sera had the same effect in each instance one of two rabbits inoculated developed a chancre

Experiments 7 and 8 The Immunization of Rabbits with Killed T pallidum Suspended in a Water in-Oil Emulsion —

Freund and his coworkers[8] and others[13 14] have shown that the antigenic activity of bacteria is often considerably enhanced if they are suspended in the aqueous phase of a water in oil emulsion This was produced by mixing an aqueous suspension of the organisms first in lanolin (or Falba) and then emulsifying the mixture in *e g* mineral oil They further showed that the incorporation of killed tubercle bacilli in the oil phase of such emulsions enhanced the sensitizing activity of the suspension

A series of treponematal suspensions was therefore prepared in which extracts of rabbits syphilitic testes, or the organisms sedimented from such extracts were emulsified in anhydrous lanolin and then in mineral oil to form suspensions containing from 47 to 370 million organisms per cc Rabbits were injected subcutaneously with varying amounts of these emulsions At varying periods thereafter specimens of blood were obtained by cardiac puncture and their Wassermann and flocculation titers determined In only one rabbit of the five (rabbit 60 66 injected with suspension E)

was there a significant increase in antibody reactive with alcoholic tissue extracts the Eagle flocculation titers on days 1, 6, 13, 23, 27, and 37 being 1½, 4, 4 8, 12, and 8 respectively

Forty-one days after the first immunizing injection, and 28 days after the last, the rabbits were inoculated intradermally and intratesticularly with 100 living organisms Every immunized animal developed darkfield positive lesions The incubation periods at the intratesticular sites did not differ significantly from those in control normal animals There was, however, an indication that at the intradermal sites, the incubation period had perhaps been decreased In the five immunized animals, the incubation period varied from 14 to 20 days, averaging 17, while in five control rabbits simultaneously inoculated, the incubation periods were 27, 38, and 45, and more than 55 days in the remaining two

In order to retest this apparent increase in skin reactivity, 24 days after the first inoculation the same animals were reinoculated intradermally at three different sites with 10^6, 10^4, and 10^2 organisms The incubation periods at the sites receiving large inocula did not differ significantly from those in control syphilitic rabbits In the sites receiving a small inoculum, however, there was again a definite indication of a reduced incubation period, the observed period varying only between 13 and 14 days in the "immunized" animals as against 23 to 27 days in the control animals

Further to test this apparent increase in sensitivity, 66 days after the last injection of the killed organisms, and 38 days after the first inoculation with living treponemata, fresh skin areas were injected with 2 million killed and live organisms A third site in each rabbit was injected with a similarly prepared control extract of normal rabbit testes Normal and syphilitic control animals were simultaneously injected The normal testis extract produced no reaction in any of the animals In one of the five immunized animals, but none of the controls, the killed organisms caused a significant erythematous reaction within 24 hours which reached a peak in 48 hours, and had disappeared after 72 hours The live organisms produced a darkfield positive lesion in 2 to 7 days, averaging 4, in the immunized animals, and in 2 to 10 days, averaging 6, in the control series

In summary, the immunization of rabbits with killed *T pallidum* suspended in water in oil emulsions after Freund, with or without the simultaneous injection of an adjuvant antigen (killed

tubercle bacilli), did not cause the development of demonstrable resistance to infection with *T pallidum* There was, however, an indication that such immunization may perhaps have sensitized some of the rabbits to *T pallidum* The incubation period on intradermal inoculation with live organisms was decreased, and one of the five experimental rabbits developed an erythematous wheal 24 hours after the injection of a heat-killed suspension of the organisms

DISCUSSION

Every one of thirty seven rabbits injected intravenously with a total of 4 4 to 38 billion *T pallidum* in aqueous suspension developed positive Wassermann or flocculation tests (complement fixation and precipitation with alcoholic extracts of beef heart) in significantly increased titer The dilution titers of these tests in the normal serum controls varied from 0 to 1 4, averaging less than 1 2, the titers in the immunized series rose to as high as 1 96 This antibody response reached its maximum levels within 2 to 3 weeks, and thereafter either remained constant during the period of immunization or, in two experiments involving the continuing injection of large numbers of organisms fell steadily in the course of the following 6 to 7 weeks

The organisms used in these experiments were derived from rabbit testicular chancres, and the suspensions of necessity contained tissue extractives The present experiments therefore do not constitute a rigorous demonstration that Wassermann and flocculation reagin is a specific antibody to *T pallidum* The possibility remains that the tissue extractives contain a haptene activated by the treponematal protein to form a complete antigen However, there are two aspects of the present experiments which make that explanation unlikely The first is the demonstration that control animals injected with extracts of normal testes, either with or without the addition of non-pathogenic Reiter spirochetes failed to develop these antibodies One would therefore have to assume either that pathogenic *T pallidum* differs qualitatively from the cultivated organisms in its ability to activate the tissue haptene to a complete antigen, or that the syphilitic testes contained a haptenic substituent not present in normal tissue The second point is the present demonstration that sedimented organisms containing only minute amounts of tissue extractives were just as antigenic as the crude chancre

emulsion from which they had been concentrated The simplest explanation of the present data is that Wassermann or flocculation reactivity induced by the injection of these organisms, and presumably also the similar "reagin" elaborated during syphilitic infection, represent an antibody response to pathogenic *T pallidum* This was the thesis originally postulated by Wassermann when he developed the complement fixation test for syphilis which bears his name, and which was apparently negated by the subsequent demonstration that alcoholic extracts of normal mammalian tissue could be used as antigen One need only assume that the treponemata and the mammalian tissues contain an immunologically related antigen The final demonstration of that fact must await either the cultivation of the pathogenic organism, or the preparation of suspensions sufficiently concentrated or sufficiently free from tissue extractives to warrant their chemical fractionation or their use in cross-absorption experiments Attempts in this direction are now in progress

Paradoxically, the 'immunized' rabbits in the present series did not regularly develop a significant resistance to infection Intradermal inoculation in some experiments with as few as 10 treponemata, regularly resulted in a typical darkfield positive primary lesion at the site of inoculation, whether the animals had been immunized with totals of 30 million organisms intradermally, 537 million organisms subcutaneously, 4 4 to 38 billion organisms intravenously, or 130 to 1040 million organisms in an oil in water emulsion, administered over periods which varied from 13 days to 4 months Recently, Magnuson, Halbert and Rosenau[15] have also reported failure to produce a significant measure of resistance to infection by the injection of pathogenic *T pallidum* suspended in oil in water emulsions of the type here used

Three of five immunized rabbits which were challenged by the intratesticular inoculation of ten organisms failed to develop a primary lesion, while every one of five simultaneously inoculated controls was infected In the one such animal tested, there had been an asymptomatic infection, the organisms having disseminated without producing a primary lesion at the site of inoculation The significance of this observation, in the light of the results after intradermal inoculation, is open to question The at best small measure of resistance to infection in these artificially immunized rabbits contrasts sharply with the fact

that in the course of an actual infection the animals develop a solid immunity, to the degree that after they have been cured inocula of many million organisms fail to produce even an asymptomatic second infection Equally paradoxical, and perhaps related to the foregoing, is the fact that the immunized animals, while developing antibodies to a non specific antigen (alcoholic extract of beef heart), failed to develop antibodies directly active against the treponematal suspension itself The organisms were *not specifically* agglutinated, the sera did not give specific complement fixation with the treponematal suspensions, and as few as ten living organisms incubated for 1 hour with a high titered (Wassermann and flocculation) serum from an immunized animal, retained their infectiousness on inoculation into a normal animal It is possible that the rabbits had been overimmunized, and were in a "negative phase" at the time they were tested for resistance to infection, or at the time their sera were tested for direct antitreponematal reactivity There is the future possibility that the surface of the organisms contains a relatively non antigenic material, and that the most effective antigen is intracellular This might explain the development of serum antibodies which cross react in high titer with non specific antigens, despite the absence of reactivity (specific agglutination, complement fixation, lysis, or protection) with intact *T pallidum* However, this would not explain the pronounced immunity which develops in the course of actual syphilitic infection, but not in rabbits immunized with killed organisms The final, if unlikely, possibility is that in none of the animals was there an antibody response to the treponemata as such, and that the Wassermann reagin was an antibody response to the small amounts of tissue extractives present in the treponematal suspension Under ordinary circumstances, those rabbit extractives are non antigenic for rabbits, even if injected simultaneously with cultured treponemata The pathogenic organisms may nevertheless possess a unique ability to activate the homologous tissue haptene to a complete antigen

Of particular interest is the fact that in one series of animals, injected with organisms suspended in an oil in water emulsion, there was a suggestion that some of the rabbits may have been sensitized to the treponemata by the preceding immunization The incubation period on intradermal inoculation with small numbers of organisms was decreased, and one of five rabbits reacted to the intradermal injection of killed organisms The pos-

ible relationship of this observation to the late manifestations of the disease, in which relatively small numbers of organisms produce a disproportionately large tissue reaction, is apparent

SUMMARY

The intravenous injection of suspensions of dead *T. pallidum* into rabbits regularly caused the appearance of Wassermann and flocculation antibodies in significantly increased titer Control suspensions of cultured treponemes (Reiter strain) added to extracts of normal testes were ineffective The probable inference is that the Wassermann and flocculation reagin elaborated during syphilitic infection is an antibody to *T. pallidum* which happens to cross-react with alcoholic extracts of mammalian tissue

The antisera did not cause the agglutination of suspensions of pathogenic *T pallidum*, living or dead, did not give specific complement fixation with those suspensions, and did not cause the living treponemata to lose their infectiousness

Animals immunized with such aqueous suspensions for as long as 4 months, or with organisms supended in a water-in oil emulsion, were not demonstrably resistant to infection As few as ten living organisms inoculated intradermally into animals "immunized' with as many as 38 billion dead treponemata regularly produced typical darkfield positive infections, and two of five animals inoculated intratesticularly with ten organisms were also infected.

The contradiction involved in the production of antibodies cross-reacting with a non-specific antigen, and the non appearance of specific antibodies against the organism used as antigen, are discussed in the text

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STUDIES ON THE ANTIGENIC CONSTITUTION OF THE REITER SPIROCHETE

By
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Studies by Beck[1] and Kolmer[2] have shown that agglutination and complement fixation tests with nonpathogenic strains of spirochetes such as Reiter, Kazan, Kroo, Nichols and Noguchi yield a higher percentage of positive reactions in sera from patients with syphilis than in normal sera The utility of such spirochetal antigens in the diagnosis of syphilis is negated by the occurrence of nonspecific reactions in a significantly higher percentage than with beef heart antigen However, it appears possible that a higher degree of specificity may be achieved with purified antigens obtained by fractionation of spirochetes Limited attempts in this direction have been made by Beck[1] and by Kolmer[2] who reported precipitin and complement fixation tests in Wassermann positive sera with a tryptic digest of Reiter *S. pallida* as antigen The present studies are a continuation and extension of this work

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The Reiter spirochetes were grown in a Brewer-type thioglycollate medium, heavily buffered with disodium phosphate, and containing no agar Ten per cent by volume of heated human serum was added Cultivation was carried out in large Erlemeyer flasks or narrow-necked bottles of 6 20 liter capacity Constant, gentle agitation of the culture throughout the entire period of growth was effected by means of a magnetic stirrer The spirochetes were harvested after 4-5 days incubation at 37° C, killed with formalin and washed repeatedly with saline in the cold (4° C) The organisms were then washed with acetone, dried, and the resulting powder was homogenously suspended in distilled water and digested with trypsin at pH 8 0 Yields of 200 240 mgs of dried spirochetes per liter of culture usually were obtained

The milky fluid resulting from digestion was centrifuged at 850 g to remove spirochetal detritus and then centrifuged at approximately 23,000 g The grey white precipitate obtained was designated fraction K, the very slightly opalescent yellow supernatant from the high speed centrifugation yielded two more serologically active fractions, L and M, after addition of absolute alcohol to final concentrations of 67 per cent and 80 per cent, respectively The K fraction was purified by three cycles of centrifugation, collecting the material sedimented at 23,000 g, but not at 850 g The L and M fractions were purified by three re precipitations with absolute alcohol at concentrations of 67 per cent and 80 per cent, respectively, in the presence of 4 per cent sodium acetate

Fig 1 presents the results of physical and chemical analyses of the three fractions It is noteworthy that K, as isolated by dialysis against distilled water, contains no sodium, while L and M, also dialyzed, contain considerable amounts The nitrogen content of K is quite low, subfractionation of a portion of K indicated that the small amount of nitrogen present is an impurity The phosphorous content varied considerably, probably as a result of incomplete purification Molisch reactions were positive in all fractions The Biuret test was negative in K only Optical and viscosity measurements could not be obtained on K because of its physical characteristics, in L and M an interesting difference is noted, i e, L is dextrorotatory and M is levorotatory Relative viscosities of the L fractions were significantly higher than those of M

Both L and M contain considerable amounts of nucleic acid as measured by ultraviolet light absorption, the larger amount

FIGURE I

Phys cal and Chem cal Propert es of Fract ons from
Trypt c D gest of Re ter *S Pall da*

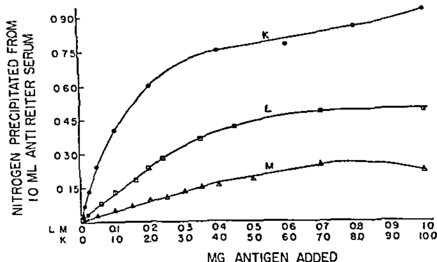
Fract on	KI	KII	LI	LII	MI	MII	L-Ie
Sod um %	0	0	4 3	5 3		6 5	
N trogen %	3 1	3 2	11 3	12 7	9 3	8 9	12 2
Phosphorus %	6 1	2 5	0 8	1 8			7 7
Mol sch	++	+	+++	+	++	++++	
B uret	0	0	+	++	++++	+	
Opt cal Rot ( )D			+49 6	+57 6	—37 1	—11 7	
3/3, H_2O 0 2% conc			1 43	2 31	1 01	1 01	
3/3, sal ne 0 1% conc			1 11	1 38	1 02	1 01	
UV absorpt on at 258 m μ 100 μ g/ml			1 061	1 311	0 574	0 744	1 280
UV ab orpt on at 235 m μ			0 562	0 627	0 370	0 402	0 394
Pptn w th ant Re ter serum	pos	pos	pos	pos	pos	pos	neg

being present in L Fraction L was subjected to electrophoresis which revealed the presence of three major components The fastest component had a mobility compatible with nucleic acid and was separated Properties of this component LIe are recorded in the last column of Fig 1 the nitrogen phosphorous ratio and character of the ultraviolet absorption curve show it to be nucleic acid

Fractions K L and M gave precipitin reactions with rabbit antisera against the whole Reiter organism (Fig 1) Fraction K behaved as a single antigen and removed almost all of the agglutinin antibody it also removed all antibody to L and M Fraction L is not completely pure in its precipitin behavior it removes about 65 per cent of the antibody reactive with K and all the antibody to M Fraction M is quite impure The immunological relationships between K L and M are shown in Table II

Sera from rabbits and humans with and without syphilitic infection have been examined by precipitin tests with fractions K and L In most instances the tests with sera from syphilitic animals contained larger amounts of precipitate than those with normal sera However in view of the fact that many normal sera contain small but definite amounts of antibody reacting with K and L the value of this reaction as a diagnostic test is questionable Only preliminary work on this aspect of the problem

Fig 1



has been accomplished Purer antigens and the testing of large numbers of sera, will be necessary before any conclusions can be drawn

We have confirmed quantitatively (Table III) the demonstration by Beck and Kolmer that Reiter spirochetes react with an antibody other than that reacting with beef heart antigen A pool of sera from three patients with secondary syphilis yielded 41 µg of nitrogen precipitated by K and had an Eagle flocculation titer of 32 u After absorption with K, the flocculation titer was unaffected, while absorption with Kahn antigen floccules resulted in a negative flocculation test, although K still precipitated 40 µg of nitrogen

TABLE II

Immunological Relations Among K L and M Fractions

Antigen used for tests	Precipitation with Rabbit Anti Reiter Serum			
	Unabsorbed	Abs with K	Abs with L	Abs with M
K	++++	+	++	+++
L	+++	—	±	+++
M	++	—	—	±
Aggl titer of whole organism	1024	16		

TABLE III

Independence of Antibody to K and Wassermann Ant body

Antigen used for tests	Precipitation and Flocculation with Human Syph Serum		
	Unabs	Abs with K	Abs with Beef Heart Antigen
K	41 µg N	0	40 µg N
Eagle	32 un ts	32 units	0

It has also been found that the K and L fractions derived from the Reiter strain react with antisera prepared against the Kazan and Nichols strains, as well as a strain of a mouth spirochete (S26) Sufficient data are not yet available to give a quantitative expression of the degree of relationship The cross reaction with the mouth spirochete would explain the presence of traces of antibody to Reiter antigens in normal sera

The information at hand shows that significant antigen relationships exist between virulent *T pallida* and the various nonpathogenic spirochetes, in confirmation of earlier work with whole organisms

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PHENOMENA OF DISEASE IN RABBITS FED CHOLESTEROL AND INFECTED WITH TREPONEMA PALLIDUM

A Preliminary Report

By

C N Frazier, C. D Boyd, E Grunwaldt, E Barker, and *M Davis**

It was our purpose to induce the localization of *Treponema pallidum* in the aorta of the rabbit damaged by the feeding of cholesterol It was thought that cholesterol might cause a lesion of the blood vessel which would be a favorable focus for the pathogenic activity of this organism

Although the experiment has not progressed far enough to allow any statement as to the possibility of achieving this objective, certain observations have been made on the nature and location of the injury resulting from the deposition of cholesterol, and on the apparent localization of syphilitic disease in one of the affected tissues, namely, the ocular iris

Without going into the details of the experiment, it is sufficient to state that 25 male rabbits were fed daily 100 gm of Purina rabbit food in the form of pellets which were coated with 0 5 gm of cholesterol in cotton seed oil Animals that were fed the cholesterol diet for 63 days developed well-defined atheromatous lesions of the aorta These first appeared on the intima distal to the larger effluent branches of the aorta and were of such a shape as to suggest a relationship with the turbulence of the blood stream at these locations The aortic arch became involved in the ascending transverse and descending portions In some animals atheromatous changes extended along the entire length of the thoracic and abdominal aorta The pulmonary arteries were also involved Less often the renal arteries showed small atheromatous lesions Of interest was the involvement of the aortic cusps and the coronary ostia The coronary arteries, too, were infiltrated and stiff In 3 animals, which were examined atheromatous changes did not extend into the cerebral blood vessels

This report is concerned chiefly with the visible lesions pro

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duced by cholesterol in the iris of the eye and with the apparent response of these lesions to syphilitic infection

At about 100 days after the feeding of cholesterol began, xanthomatous deposits were found in the irides of 6 rabbits Four of the animals were albinos and 2 were pigmented breeds By the 120th day of feeding the lesions were well developed and encircled the iris at the periphery, extending half way across its width toward the pupillary margin

After 119 days on cholesterol, the animals had reached the limit of tolerance, and the feeding of cholesterol was stopped At that time the total cholesterol in the blood ranged from 0 648 gm to 3 438 gm per cent The normal range of total cholesterol in the blood before treatment was from 0 032 gm to 0 216 gm per cent After the feeding of cholesterol was stopped, the cholesterol content of the blood fell to within normal limits in from 49 to 150 days As time went on the xanthomatous lesions of the iris showed evidence of resorption This was 6 months after feeding had stopped One hundred sixteen days after discontinuing the cholesterol, and when the xanthomatous lesions were at maximum size, 14 animals were fed cholesterol and 15 animals not fed cholesterol were inoculated intratesticularly with the Nichols' strain of *Treponema pallidum* All inoculated animals developed an orchitis The mean incubation period of the cholesterol fed rabbits was 14.9 days and that of the unfed controls was 18 1 days

Shortly before the development of metastatic orchitis of the uninoculated testis, 4 of the albino rabbits with xanthomatous irides showed signs of iritis There was prominent vascularization of the iris in and around the xanthomatous lesions, and prominent circumcorneal congestion adjacent to the lesions The 2 animals with pigmented irides and xanthomatous deposits showed no circumcorneal congestion There was no evidence of iritis in the animals not fed cholesterol except that in one animal a transient congestion around the cornea was observed

The mean time of appearance of iritis was 34 5 days after inoculation Except in one animal, this was somewhat sooner than the time at which signs of a metastatic orchitis appeared All the cholesterol fed rabbits developed a metastatic orchitis at a mean of 44 4 days after inoculation Only 7 of the 15 animals not fed cholesterol had a metastatic orchitis In these rabbits the mean time of appearance was 54 7 days after inoculation In the 4 animals that developed iritis, the lesion persisted for

14, 36, 43, and 209 days, respectively, and healed spontaneously without any gross residual scarring

Another series of 4 rabbits were fed cholesterol in cotton seed oil in the same amount after they had been infected with *Treponema pallidum* for 324 days All the demonstrable lesions of syphilis had long since disappeared In one rabbit, an albino, xanthomatous lesions of both irides were present 60 days after the feeding of cholesterol began Ninety days later, which was 477 days after inoculation and well beyond the usual time of active syphilitic lesions, an iritis appeared in both eyes In one eye the entire iris was involved and the aqueous humor was cloudy There was no gross evidence of vascularization of the cornea, but the circumcorneal vessels were deeply congested to a width of from 2 to 3 millimeters It is believed that the iritis was syphilitic in nature

Although in no case has an attempt yet been made to isolate the organisms from the iris and ciliary body of the affected eyes, or from the eyes of animals that did not develop iritis, it would appear from the observations just described that the xanthomatous lesions served to affect the response of these tissues to syphilitic infection Whether a similar response occurred in other tissues injured by cholesterol remains to be determined

EXPERIMENTAL MOUSE SYPHILIS
I ORGAN DISTRIBUTION OF THE INFECTIOUS AGENT

By

Paul D Rosahn, Boris Gueft and *Catharine L Rowe**

The standard method for the assay of penicillin preparations in the treatment of experimental syphilis employs the rabbit as the test animal and requires a total elapsed time of approximately nine months before results are available Recently Rake[1] and Turner[2] and their associates have reported on assay methods which not only shorten this period, but utilize smaller quantities of the test drug for the complete assay In a consideration of this problem, it was believed that the mouse could probably be substituted for the rabbit as the assay animal Accordingly a study of the literature on mouse syphilis was made in order to obtain background for the development of a mouse assay method From this review[3] it became apparent that there existed numerous conflicting reports and unconfirmed findings, and that, therefore, it would be necessary to restudy certain aspects of the biology of syphilitic infection in the mouse The present report describes our findings on the organ distribution of the infectious agent of syphilis in the inoculated mouse

Kolle and Schlossberger[4,5] in 1926 first discovered that the mouse develops an asymptomatic infection following inoculation with virulent treponemes This infection is readily demonstrated by the injection of an emulsion of tissue from the asymptomatic mouse into the testicle or skin of a rabbit, at which site a syphiloma rich in treponemes makes its appearance after a short incubation period Whether or not the mouse tissue harbors active treponemes, or granular or invisible variants of treponemes, is not the concern of this communication This aspect of the problem has already been reviewed elsewhere[3,6] We ourselves have never identified treponemes on darkfield examination of mouse material proved by rabbit subinoculation to be infectious

Almost all of the previous investigators have studied the dis-

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tribution of the agent of syphilis in mouse tissues by rabbit subinoculation with emulsions of pooled tissue obtained from several mice This technique effectively demonstrates the presence or absence of the infectious agent in the pooled material, but gives no indication of the proportion of infected mice in an inoculated group Because this information was of major import in the development of a bioassay method, our study was based on a uniform technique, i e, the inoculation of an organ emulsion from a single mouse into a single rabbit If the rabbit developed a darkfield positive lesion at the site of inoculation, the infectious agent of syphilis was considered to have been demonstrated in the mouse organ constituting the inoculum

Material and Methods

Animals All mice were of the white hybrid variety, the so called 'Swiss" type Male mice were employed predominantly Most of them were sexually mature of varying ages In a few tests newborn mice were inoculated and in others, mice one, two and three weeks old were utilized The rabbits were of mixed breeds, all males, weighing from five to seven pounds at the time of inoculation

Housing and Feed Originally small groups of mice were maintained in a single cage, but later in the investigation it was found necessary to segregate them in individual compartments They were fed commercial food pellets and a free supply of water Rabbits were housed in individual cages, and received compressed food pellets and an adequate amount of water All animals were kept in large well illuminated rooms, maintained at a temperature of 65 70° C

Mouse Inoculation Rabbits infected with the Nichols strain of *T pallidum* served as the source of the infectious agent Active testicular syphilomas from routine passage rabbits were excised, minced, and ground in a mortar with saline The resulting emulsion was diluted so as to contain from 2-4 treponemes per darkfield Quantitative enumeration of the treponemes was not done The standard inoculum was 0 2 or 0 3 cc of this emulsion Two routes of inoculation were studied, the intraperitoneal and the subcutaneous Other inoculation methods, intracerebral, intratesticular, intravenous, and intradermal were not employed, because they involved procedures not readily adaptable to a bioassay technique Following inoculation all mice were carefully observed for the development of localized specific lesions None was noted

RABBIT INOCULATION. After the lapse of varying periods following inoculation, the mice were sacrificed by ether inhalation Five tissues were studied. 1. *Blood*—Immediately after death the pericardium was opened, and blood aspirated from the heart with needle and syringe An average of about 0 5 cc was obtained, and this was inoculated into one or both testicles of a single rabbit 2 *Brain*—The scalp was reflected and the calvarium lifted off the brain This was then removed, placed in a mortar, ground up with about 1 cc of saline, and 0 5 cc injected into each testicle of a rabbit The entire brain of one mouse thus constituted the inoculum for one rabbit 3 *Skin*—The dorsum of the mouse was shaved closely, swabbed with alcohol, and a strip of skin usually measuring about 1 x 3 cm was excised This was ground in a mortar with about 1 cc of saline, and half of the resulting emulsion was inoculated into each testicle of a test rabbit The excised skin site was taken at random, and had no relation to the original site of the mouse inoculation 4 *Spleen*—Under sterile conditions the spleen was excised, ground in a mortar with approximately 1 cc of saline, and the resulting emulsion was inoculated in its entirety into the right and left testicles of a single rabbit 5 *Lymph Nodes*—All available lymph nodes were removed from the mouse under examination These included the inguinal, periaortic, axillary and subclavian groups They were then pooled in a mortar, ground with 1 cc of saline, and the entire emulsion inoculated into the right and left testicles of a single rabbit In some experiments all five tissues enumerated above were obtained from a single mouse and each tested by inoculation into five different rabbits In other experiments two or three tissues from a single mouse were checked for invasion by subinoculation into different rabbits In most of the experiments, however, a single mouse organ was subject to the rabbit test for the presence or absence of the infectious agent

Following inoculation with mouse tissue as described above the test rabbits were observed by frequent clinical examination All suspicious testicular lesions were subjected to darkfield examination When this revealed treponemes, the mouse tissue forming the inoculum was considered to have been invaded by the infectious agent of syphilis All negative rabbits were observed for 90 days, at which time, if still negative, the experiment was terminated

Results

1 Subcutaneous Inoculation In Table I are shown the results obtained following subcutaneous mouse inoculation The duration of mouse infection varied from 27 to 90 days before the excised organs were subinoculated into rabbits as a test of infectivity The lymph nodes from all of 18 mice produced lesions in rabbits, but only 7 of 34 mice, or 21 per cent, had brains which gave evidence of invasion Skin showed a high proportion of positive results, 16 out of 20, or 80 per cent, while 12 of 21 mice or 57 per cent had spleens, and 3 out of 11 mice or 27 per cent had blood which produced darkfield positive lesions in subinoculated rabbits It should be noted that at 45 days

TABLE I

Distribution of Infectious Agent of Syphilis in Mouse Organs as Determined By Intra Testicular Subinoculation Into Rabbits Mice Inoculated Subcutaneously *

Duration of Mouse Infection	Mouse Organ				
	Blood	Skin	Spleen	Brain	Nodes
Days					
27			—	1/2	2/2
45–49	3/11	9/10	7/12	1/20	14/14
57	—	—	—	0/2	2/2
90		7/10	5/9	5/10	—
27 90	3/11 27 3%	16/20 80 0%	12/21 57 1%	7/34 20 6%	18/18 100%

* In this and subsequent tables the denominator of all fractions indicates the number of mice tested and the numerator the number of positive mice

following inoculation, only 3 out of 11 mice had blood which produced lesions in rabbits even though the lymph nodes of all of these mice harbored the infectious agent of syphilis

The distribution of positive lesions is homogeneous in those categories where comparisons can be made within the vertical columns of the table, except in the case of the brain In this instance, 2 out of 24 mouse brains tested in from 27 to 57 days after subcutaneous inoculation were positive, as compared with 5 of 10 mouse brains tested after an incubation period of 90 days (Table III) The difference between these two groups is significantly different (Chi-square = 7 8, P = 0 01), indicating that within the time limits studied, increasing the duration of

infection in the subcutaneously inoculated mouse increased the probability of brain invasion With regard to spleen and skin, the only two other mouse organs for which comparative data are available, there was no indication that increasing the infectious period in the mouse affected the proportion of positive results

2 INTRAPERITONEAL INOCULATION · Table II summarizes our findings with intraperitoneally inoculated mice Of 35 mice tested in from 10 to 90 days following injection, 27 or 77 per cent had blood which produced darkfield positive lesions in rabbits Mouse skin, spleen, brain and lymph nodes were studied following mouse infection varying for 10 to 169 days With skin, 30 out of 62 tests were positive, or 48 per cent Of 55 mice whose spleens were inoculated into rabbits, 39 or 71 per cent produced darkfield positive lesions Of all organs studied, mouse brain was least frequently invaded by the infectious agent of syphilis Only 31 per cent, or 27 out of 88 brains, were positive Lymph nodes on the other hand, were most frequently invaded, for 91 out of 98 tests or 93 per cent were positive

TABLE II

Distribution of Infectious Agent of Syphilis in Mouse Organs as Determined by Intratesticular Subinoculation into Rabbits Mice Inoculated Intraperitoneally

Duration of Mouse Infection	Mouse Organ				
	Blood	Skin	Spleen	Brain	Nodes
Days					
10-14	2/4	2/4	4/4	1/17	15/16
20-33	5/6	4/6	6/6	1/6	11/11
45-57	8/10	10/29	14/27	7/26	9/10
66-90	12/15	12/19	13/19	3/19	29/30
100-169		2/4	2/4	15/20	28/31
10-169	27/35 77.1%	30/62 48.4%	39/55 70.9%	27/88 30.7%	91/98 92.9%

The duration of mouse infection, within the time limits shown in Table II, did not alter the frequency of positive results with any organ except brain In this instance 12 of 68 brains tested in from 10 to 90 days after intraperitoneal inoculation were positive, or 18 per cent, in contrast to 15 positive brains out of

20 tested in from 100-169 days after mouse injection, or 75 per cent (Table III) These two values are significantly different (Chi-square = 24 15, P = 0 01). *As with subcutaneous inoculation*, increasing the period of time elapsing between intraperitoneal inoculation and the rabbit test, increased the probability of mouse brain invasion

TABLE III

Frequency of Mouse Brain Invasion Following Subcutaneous or Intraperitoneal Inoculation

Subcutaneous Inoculation			Intraperitoneal Inoculation		
Duration of Mouse Infection	Result		Duration of Mouse Infection	Result	
Days		%	Days		%
27 57	2/24	8 3	10-90	12/68	17 6
90	5/10	50 0	100 169	15/20	75 0

3 Comparison of Subcutaneous and Intraperitoneal Routes of Inoculation It is now possible to compare the results of subcutaneous and intraperitoneal inoculation, as shown in Table IV All of the previously cited findings are here included, with the exception of those for brain In this instance there have been excluded the observations on subcutaneously inoculated mice whose brains were tested at 90 days, and of intraperitoneally inoculated mice whose brains were checked for invasion at 100-169 days These values have been omitted from the table because as has been shown, they significantly differ from the results obtained at shorter intervals following inoculation A comparison of the subcutaneous and intraperitoneal routes of inoculation shows no significant differences in the proportion of positive results with blood, spleen, brain or lymph nodes A difference of significance does, however, exist with skin Of the subcutaneously inoculated mice whose skin was tested, 16 out of 20 or 80 per cent gave positive results, as contrasted to 30 out of 62 mice or 48 per cent whose skin was checked for invasion following intraperitoneal inoculation This difference is significant (Chi square = 6 19, P = 0 01-0 015) Subcutaneous inoculation is thus the procedure of choice to secure skin invasion

TABLE IV

Distribution of Infectious Agent of Syphilis in Mouse Organs   Comparison of of Two Routes of Inoculation

Mouse Organ	Mode of Inoculation	Duration of Mouse Infection	Result	
		Days		%
Blood	Subcutaneous	45	3/11	27 3
	Intraperitoneal	10–90	27/35	77 1
Sk[illegible]	Subcutaneous	45–90	16/20	80 0
	Intraperitoneal	10–169	30/62	48 4
Spleen	Subcutaneous	45 90	12/21	57 1
	Intraperitoneal	10–169	39/55	70 9
Brain	Subcutaneous	27 57	2/24	8 3
	Intraperitoneal	10–90	12/68	17 6
Nodes	Subcutaneous	27–57	18/18	100
	Intraperitoneal	10 169	91/98	92 9

I INCUBATION PERIOD Table V summarizes the incubation period in the rabbit following intratesticular inoculation of various tissues derived from subcutaneously inoculated mice The incubation period is here defined as the interval between intratesticular inoculation of mouse material, and the development of a darkfield positive lesion in the rabbit Analysis shows no significant heterogeneity between the different classes of mouse organs The mean incubation period for the 56 positive mouse

TABLE V

The Interval (Incubation Period) Between Inoculation and the Development of Darkfield Positive Lesion in the Rabbit Following Intratesticular Inoculation With Infected Mouse Tissue Mice Inoculated Subcutaneously

Mouse Organ	No	Incubation Period in Rabbit		
		Minimum	Maximum	Mean
		Days	Days	Days
Blood	3	39	53	45
Skin	16	[illegible]0	50	36
Spleen	12	35	54	39
Brain	[illegible]	30	51	37
Nodes	18	27	47	38
Total	[illegible]	27	54	38

tissues was 38 2 ± 2 56 days The shortest incubation period was 27 days, obtained with lymph nodes, and the longest was 54 days following inoculation with mouse spleen

The incubation period in the rabbit when subinoculated with various organs from intraperitoneally infected mice is shown in Table VI The shortest incubation period was 21 days with lymph node material Of the 214 observations 8 or 3 per cent exceeded 60 days One of these was 84 days with blood as the inoculum, two (67 and 69 days) resulted from skin inoculation, two (66 and 70 days) followed spleen inoculation, one (62 days) resulted from brain inoculation, and two (81 days each) followed inoculations with lymph nodes With 91 lymph node tests, only 2 per cent resulted in incubation periods exceeding 60 days

TABLE VI

The Interval (Incubation Period) Between Inoculation and the Development of Darkfield Positive Lesions in the Rabbit Following Intratesticular Inoculation With Infected Mouse Tissue
Mice Inoculated Intraperitoneally

Mouse Organ	No	Incubation Period in Rabbit		
		Minimum	Maximum	Mean
		Days	Days	Days
Blood	27	33	84	41 3 ± 1 85
Skin	30	29	67	39 3 ± 1 71
Spleen	39	33	0	40 7 ± 1 35
Brain	27	31	62	37 6 ± 1 45
Nodes	91	21	81	33 9 ± 0 83
Total	214	21	81	37 3 ± 0 61

The mean incubation period ranged from 33 9 days with lymph nodes, to 41 3 days with blood There were no significant differences among the values for blood skin, spleen, or brain, but all of these significantly exceeded the value for lymph nodes which gave the shortest mean incubation period

5 Pooled Organs As has been indicated, our primary objective in these studies was to develop a mouse test for the bioassay of penicillin One such technique now seemed feasible The infectious agent of syphilis had been demonstrated in the skin of a high proportion of mice inoculated subcutaneously A test of infectivity of a subcutaneously inoculated mouse could now be done, employing a portion of its skin obtained by biopsy

If this were positive, i e, if it produced a darkfield positive lesion in the rabbit, the mouse could be treated, and subsequently another section of its skin could be put to the rabbit test to determine its infectivity following therapy Although technically feasible this procedure was not desirable because it would involve a prolonged period of observation, approaching that required in the orthodox rabbit assay method

It was noted that all of 25 mice were shown to be infected as evidenced by a positive result in the rabbit with one or another of three tissues tested from each mouse, i e, blood, spleen and lymph nodes A group of 30 mice were therefore further studied At 45 51 days following intraperitoneal inoculation, the mice were sacrificed and the excised lymph nodes and spleen of each were ground in a mortar with blood from the same mouse as the emulsifying agent Half of the resulting emulsion from each mouse was inoculated into each testicle of a single rabbit Darkfield positive lesions developed in only 23 of the thirty test animals Because these results did not nearly approach our success with lymph nodes the use of pooled organs was discarded as a test for cure following penicillin therapy

6 Asymptomatic Infection in Rabbits Rabbits inoculated with mouse organ emulsions were frequently clinically negative even though the rabbit test with other organs from the same mouse produced clinical and darkfield positive lesions It was obviously desirable to test the presence of asymptomatic

TABLE VII

Results of Popliteal Lymph Node Subinoculations From Rabbits Which Failed to Develop Clinical Lesions Following Intratesticular Inoculation With Various Organs From Infected Mice

Mouse Organs	Route of Mouse Inoculation		Total Rabbits Clinically Negative	Results of Subinoculation of Popliteal Lymph Nodes From Clinically Negative Rabbits	
	Subcutaneous	Intraperitoneal		Negative	Positive
Skin	1	14	15	15	0
Spleen	4	7	11	11	0
Brain	9	6	15	15	0
Nodes		1	1	1	0
Pooled Blood Spleen Nodes		6	6	6	0
Total	14	34	48	48	0

infection in these clinically negative rabbits As shown in Table VII, 48 such animals which had been inoculated with a variety of mouse tissues were further studied The popliteal lymph nodes were removed, ground with a 50 per cent saline-rabbit serum mixture, and the entire emulsion inoculated into a pair of rabbits In not a single such test was a clinical or darkfield positive lesion produced in the subinoculated rabbits This negative result gave convincing assurance that under the conditions of our experiment when a clinically demonstrable lesion failed to develop in the rabbit at the site of inoculation, the inoculated rabbit was in fact not infected

7. AGE AND SEX OF INOCULATED MICE Most of the mice in these investigations were males, but the few female animals utilized were as easily infected as were their brothers Moreover age conferred no immunity Mice of all ages including newborn animals were inoculated with success as shown by subsequent transfer of tissues into rabbits The organ distribution of the infectious agent was apparently not influenced by either age or sex

DISCUSSION

The studies herein described differ from previous investigations of a similar nature in two important respects which are in reality dual aspects of a single principle Earlier investigations employed pooled material from several infected mice for subinoculation into more than one rabbit as a test of infection Either of these procedures could conceivably reduce the number of organisms in the final inoculum, the first by adding tissue from mice in which infection had actually not been established, to tissue from mice with bona fide infections, and the second, by dividing the tissue emulsion among several rabbits If both of these diluting factors were operative in a single experiment, the minimal infectious dose of treponemes might not be present in the rabbit inoculum, and a negative test would result The technique followed in the reported studies effectively eliminated both of these sources of error The entire organ—brain, spleen, lymph nodes, blood, or a large segment of skin—from a mouse under test was emulsified, and the entire emulsion inoculated into a single rabbit Thus if the minimal infectious number of organisms were actually present in the mouse organ, a lesion in the test rabbit would be expected to develop If infection failed to develop in the test rabbit, it is a feasible presumption that the mouse organ constituting the inoculum did not contain the mini-

mal infectious number of organisms Magnuson and Eagle[7] have found that almost certainly two and frequently a single treponeme will produce a darkfield positive lesion in the rabbit, and unpublished observations of our own confirm their findings What constitutes the minimal infectious number of treponemes for the mouse is not within the scope of the present report—investigations along these lines are currently being pursued—but it appears that herein lies the solution of the longstanding controversy as to the existence of a granular or invisible phase of the treponeme

Infection could easily be established in mice of both sexes and all ages through subcutaneous or intraperitoneal inoculation with virulent rabbit syphiloma emulsions The route of inoculation exerted no influence on the frequency of invasion of spleen, brain or lymph nodes, but the subcutaneous technique produced a significantly higher incidence of invasion of skin than did the intraperitoneal route Brain was the organ least frequently invaded, while lymph nodes gave evidence of invasion in almost every instance

The duration of infection in the mouse did not notably influence the proportion of successful transfers to rabbits except in the case of mouse brain In this instance, both subcutaneously and intraperitoneally inoculated mice showed a higher incidence of brain invasion after long infection than after short infection This finding suggests that in mouse brain there is either active multiplication of the infectious agent until the threshold of the minimal infectious dose for rabbits is reached, or alternatively, that the infectious agent slowly migrates to the brain from other sites, until ultimately the minimal infectious number for rabbits is attained therein Neither of these two hypotheses is susceptible of proof with the evidence at hand

The incubation period in the rabbit following successful inoculation with mouse tissue was variable, but the outstanding characteristic was its comparatively long duration With active treponeme emulsions from rabbit syphilomas, the average incubation period in our hands[5] has been 20 days Mouse tissues gave average incubation periods varying from 34 to 45 days If incubation period is a good index of the number of treponemes in the emulsion and there is evidence[7] that this is so, the prolonged incubation period in rabbits following inoculation with mouse tissue suggests the presence of comparatively few organisms in the inoculum Furthermore this generalization suggests

that mouse lymph nodes harbor more organisms than other tissues, because the incubation period following inoculation with lymph nodes was significantly shorter than that resulting from the inoculation of any of the other four tissues studied

Eagle and his associates[9] have cited evidence which casts grave doubt on the existence of an asymptomatic primary syphilitic infection in the rabbit, and our own findings are in precise agreement with theirs Of the 48 rabbits which failed to develop a lesion at the site of inoculation, all had negative popliteal lymph nodes on transfer to rabbits These three different series offer conclusive evidence that if careful and frequent clinical examination of inoculated rabbits fails to disclose a lesion within 90 days, the rabbit is not infected An extension of these findings to man suggests that syphilis d'emblee is non existent

Summary and Conclusions

1 The mouse could be infected with *T pallidum* at all ages from birth to maturity
2 Clinical lesions were never noted in several hundred mice inoculated intraperitoneally or subcutaneously, nor were treponemes ever seen in innumerable darkfield preparations from mouse tissue which on transfer to rabbits produced active lesions containing treponemes
3 All of five mouse organs tested—blood, skin, spleen, brain and lymph nodes—harbored the infectious agent of syphilis following intraperitoneal or subcutaneous inoculation with *T. pallidum*
4 Following either method of inoculation, mouse lymph nodes were most frequently and mouse brain least frequently invaded by the infectious agent of syphilis
5 The skin of subcutaneously inoculated mice was more frequently invaded after subcutaneous than after intraperitoneal injection
6 The frequency of invasion of mouse brain was greater after long periods of infection than after short periods, as a result of either intraperitoneal or subcutaneous routes of inoculation
7 The mean incubation period in the rabbit after successful intratesticular inoculation with infected mouse tissue varied from 34 to 45 days Inoculation with lymph node material resulted in the shortest mean incubation period
8 Asymptomatic primary infection in the rabbit was not observed

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THE RELATIVE INFECTIVITY OF BLOOD AND CEREBROSPINAL FLUID IN SECONDARY SYPHILIS

By

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Invasion of the central nervous system of the human host by *Treponema pallidum* is related to the primary dissemination of the organism by the blood. In 1913, Uhlenmuth and Mulzer[1] showed that the blood of patients with primary or secondary syphilis was infectious for the rabbit. Of 55 cases of primary or secondary syphilis, the blood of 43, or 78.2 per cent, when injected into the rabbit's testis in amounts of 2.0 cc., produced a syphilitic orchitis.

Within recent years it has been demonstrated that the dissemination of spirochetes in the blood may occur in man even before the appearance of any clinically detectable lesion of syphilis[2]. In one instance the blood of a donor transmitted a virulent infection to the recipient 20 days before the appearance of a chancre on the penis of the donor and at a time when the serological tests for syphilis on the donor's blood were negative[3].

In 1906, Hoffman first found that the cerebrospinal fluid of a syphilitic man was infectious for the ape[4]. Since then several studies have shown the incidence of *Treponema pallidum* in the spinal fluid of man during the early stages of syphilis when the fluid was normal on examination by the usual laboratory tests. In three such studies in which the technique of testicular inoculation of the rabbit was used, from 15 to 20 per cent of the inoculations gave positive results[5-7]. In each case the spinal fluid was apparently normal with respect to cells, protein, complement fixation, and colloidal gold or mastic reaction.

In the last investigation of the problem by Chesney and Kemp, the spinal fluids of 34 patients were studied[8]. All patients had one or more clinical signs of early syphilis and positive serological tests on the blood. None had any demonstrable abnormality of the spinal fluid, nor did they have any physical sign of neurological disease. The duration of infection was from 3 to 6 months, and secondary manifestations of disease had been pres-

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ent for from 1 day to 10 weeks Nine of the patients were white and 25 were Negroes, 18 were males and 16 were females

Positive animal inoculations were obtained with the spinal fluid of 5, or 14 7 per cent, of the 34 patients Only fluids with cell counts below 9 per cu mm. were used All other tests were negative, including complement fixation, which was made with 1 0 cc of spinal fluid From 0 75 to 3 0 cc of fluid were used for the inoculation of each rabbit There was no observable relation between the occurrence of positive inoculations and any particular type of secondary clinical phenomenon of the disease

As dissemination of the organisms by the blood takes place very soon after infection, it must be assumed that invasion of the central nervous system likewise occurs early in the infection It is obvious that once *Treponema pallidum* enters the blood stream it necessarily is carried to the brain and spinal cord, and to the meninges Just how soon, and with what frequency, the organisms leave the blood to become localized in the extravascular tissues of the nervous system is unknown Some indication of this may be had in the frequency with which *Treponema pallidum* is present in the cerebrospinal fluid concurrently with its presence in the blood stream early in the disease It is with this aspect of the problem that we were interested

The study which this paper reports was made in Peiping, China, on Chinese patients observed at the Peiping Union Medical College during the years from 1933 to 1941, inclusive There was a total of 50 patients, however, the animals inoculated with material from 4 of them were casualties of war and no information on them is available This leaves to be described the results of the study on the blood and spinal fluid of 46 patients, of whom 38 were males and 8 were females

At the time the inoculations were made, all patients presented active signs of secondary syphilis and 13 of them also had a primary chancre There were localizations of disease in the integumentary system in 44 patients and in the skeletal system in 14 patients One patient also had iridocyclitis and another laryngitis In no case was there any sign of neurological disease The complement fixation (Kolmer) and flocculation (Kahn) tests were positive in all patients In 29 cases, darkfield examination of cutaneous lesions was positive for *Treponema pallidum* In the remaining cases the lesions were not suitable for darkfield examination or no organisms could be found

Forty patients had received no treatment for syphilis There

were 6 who had been treated at the time of the chancre with from 1 to 3 injections of neoarsphenamine Subsequently, at intervals of from 2 to 16 months, all 6 patients developed a clinical relapse in the integumentary system, and, in addition, 3 patients also had lesions of the skeletal structures, and one had an iridocyclitis Four of the 6 patients had minimal abnormalities of the spinal fluid, but no anatomical sign of neural disease All the clinical relapses were in males Virulent organisms were isolated from the blood of each patient with clinical relapse and from the spinal fluid of two patients, each with minimal abnormalities of the fluid

METHOD OF INOCULATION For the purpose of isolating *Treponema pallidum* from the blood and spinal fluid, the following method was employed Venous blood was withdrawn from each patient and immediately 1 0 cc of blood was injected into each testis of two rabbits In like manner and in the same amount, two rabbits were inoculated with spinal fluid The inoculated animals were observed at frequent intervals of time, never less than once a week for 90 days or until signs of orchitis developed Upon the appearance or orchitis, material aspirated from the testis was examined by darkfield illumination for the presence of *Treponema pallidum* An inoculation was considered positive only when treponemes were found by this technique

When no organisms could be demonstrated microscopically in the enlarged testis, the testis was removed aseptically, emulsified in a sterile isotonic solution of sodium chloride, and 1 0 cc of the emulsion injected into one testis of each of two rabbits These animals were observed for at least 90 days before being discarded as normal

In the event that no orchitis developed after a period of 90 days in the animals originally inoculated with blood or spinal fluid, both popliteal lymph nodes were removed aseptically under ether anesthesia and emulsified in sterile isotonic sodium chloride solution The entire emulsion was divided and half was injected into one testis in each of two rabbits These animals were in turn observed for at least 90 days, or until an orchitis developed

In two instances in which animals of the first transfer developed doubtful evidence of syphilis the involved testis was removed and emulsified, and 1 0 cc of the emulsion injected into a testis of each of another two rabbits

When the animals inoculated with blood or spinal fluid failed

to develop orchitis, the chances of demonstrating organisms by transfer of tissues were small In 14 cases in which either testis or lymph node was transferred from rabbits inoculated with blood only 2 resulted in establishing a demonstrable infection in the rabbit Out of 37 transfers from rabbits inoculated with spinal fluid and failing infection, only 3 tissue transfers gave positive results In one of these cases organisms were observed only after the second transfer of testicular material The animal of the original inoculation had developed an orchitis from which no *Treponema pallidum* could be found on darkfield examination

Results of Inoculations

Infectivity of Blood The blood from 46 patients was tested for infectivity Thirty-five, or 76 1 per cent, of the inoculations produced an infection in rabbits The original inoculation gave positive results in 33 cases The period of incubation of the disease in these animals is shown in Table I

TABLE I

Period of Incubation of Syphilis in Rabbits Inoculated With Blood or Spinal Fluid

Incubation Period	Animals Infected Inoculum	
Days	Blood No	Spinal Fluid No
30–39	1	0
40–49	10	0
50–59	23	1
60–69	10	4
70–79	4	1
80–89	1	1
90–99	1	0
Total	50	7

Positive inoculations were equally distributed between male and female patients All patients in clinical relapse had infectious blood

The distribution of positive inoculations with respect to the duration of infection in the patient, as measured from the appearance of the chancre, is given in Table II The blood of one female patient was infectious for the rabbit 10 months following the chancre In this case condylomatous lesions of the skin had been present for 7 months at the time the inoculation was

TABLE II

Distribution of Positive Inoculations and Abnormal Spinal Fluids According to the Duration of Syphilis in the Patient

Duration of Infection	Cases	Inoculations		Status of Spinal Fluid		
		Blood	Spinal Fluid			
		Positive	Positive	Normal	Abnormal	Bloody
Months	No	No	No	No	No	No
1–2	14	10	0	12	2	0
3–4	15	12	4	10	3	2
5–6	5	5	2	3	1	1
7 8	5	4	3	3	2	0
9 10	1	1	0	1	0	0
11 12	1	0	0	1	0	0
13	1	1	0	1	0	0
Unknown	4	2	0	4	0	0
Total	46	35	9	35	8	3

made In another case, that of a male patient who was in clinical relapse, the blood was infectious 36 months following the appearance of the chancre, and 16 months after the administration of 3 doses of neoarsphenamine The spinal fluid of the two patients was normal and did not infect rabbits There were 6 patients with darkfield positive lesions of the skin whose blood did not transmit syphilis

INFECTIVITY OF CEREBROSPINAL FLUID The spinal fluids of 46 patients were transferred to the testes of rabbits There were 9, or 19 6 per cent, positive inoculations, all from the fluids of male patients In 7 instances the animals directly inoculated with spinal fluid developed syphilis in from 56 to 82 days after the inoculation (Table I)

Of the 46 spinal fluids which were examined, 35 were normal to routine laboratory tests, including the cell count, the protein content, and the complement fixation and colloidal mastic reaction The remainder showed minimal abnormalities in the number of cells and in the content of protein, except that 3 fluids also gave a positive colloidal mastic reaction, and 1, a weakly positive complement fixation reaction (2 plus in 0 5 cc of fluid) Cell counts above 10 per cu mm with or without an increase of protein or above 5 per cu mm with an increase of protein were considered abnormal Five of the normal and 4 of the abnormal spinal fluids produced a syphilitic orchitis in rabbits Among the

infectious abnormal fluids was the one showing the greater deviation from the normal

There was no significant correlation between spinal fluids which were infectious for animals and any particular kind of clinical lesion The nearest approach to such a relationship was in the patients with alopecia There were 10 cases of alopecia among the 46 patients in the sample Four of the 10 patients showing a loss of hair had infectious spinal fluids In other words 40 per cent of patients with alopecia and 13 8 per cent of those with no alopecia had fluids which established an infection in rabbits The difference of 26 2 per cent however is not statistically significant being within the range of sampling error (x/δ = 1 59)

There has been one previous report on a study of the cerebrospinal fluid in syphilitic Chinese patients The study was made during 1931 1931 by Pearce Hu and Mu at the Peiping Union Medical College[9] It is of interest to observe that in none of 40 patients who were studied was the spinal fluid infectious for rabbits Included in the number examined were 10 patients with early active manifestations of syphilis who had never been treated for this disease All had normal spinal fluids There were also 5 patients in neurorelapse 3 of whom showed an elevated cell count in the spinal fluid The technique of inoculation the size of the inoculum and the period over which the animals were observed conformed to the practice in the present study Both studies were made in the same laboratory On the basis of our experience one might have expected to find that 2 or 3 of the spinal fluids were infectious

RELATIVE INFECTIVITY OF BLOOD AND SPINAL FLUID From the results of the inoculations it is apparent that infection of the blood in early syphilis does not indicate infection of the spinal fluid However infection of the spinal fluid is accompanied by infection of the blood At least this was true of all patients in this study who had infectious spinal fluids

It is not to be assumed that the results of inoculation are absolute in their indication of the presence of organisms in either the blood or the spinal fluid The relative probability of infectivity of the blood and spinal fluid is unknown Under optimal conditions one is inclined to the belief that there probably are more treponemes per unit volume in the blood than in the spinal fluid The infectivity of these tissues in any case would depend primarily upon the number of organisms present and upon the

susceptibility of the rabbit to the first inoculation with organisms unaccustomed to the new host It might be suspected that the blood of all patients in the active secondary stage of syphilis carries spirochetes, although it is possible that the period of greatest infectivity precedes the appearance of metastatic lesions

If it is assumed that the blood of patients whose infection is not over 2 months' duration contains a reasonable number of organisms and is infectious the blood of all 14 patients of this study in this period of the disease should have produced syphilis in the inoculated rabbits As it was, only 10 of the specimens did so This would suggest that the experimental error was about 25 to 30 per cent Assuming, further, that the same error would apply to the infectivity of the spinal fluid, the expected number of positive inoculations would be 12, or 26 per cent of all the tested fluids This theoretical figure may not be too inaccurate, since there were 3 fluids with minimal abnormalities in the number of cells and in the content of protein that did not infect rabbits

This study offers no evidence as to the time after infection when invasion of the spinal fluid occurs It is of interest, how-

TABLE III

Distribution of Positive Inoculations and Abnormal Spinal Fluids According to the Duration of Secondary Manifestations of Syphilis in Patients

Duration of Secondary Lesions	Cases	Inoculations		Status of Spinal Fluid		
		Blood	Spinal Fluid			
		Positive	Positive	Normal	Abnormal	Bloody
Weeks	No	No	No	No	No	No
1–2	15	11	2	13	2	0
3–4	9	7	0	5	3	1
5–6	2	2	1	2	0	0
7–8	13	9	4	7	4	2
9–10	0	0	0	0	0	0
11–12	2	2	2	1	1	0
13–14	0	0	0	0	0	0
15–16	1	1	0	1	0	0
17–18	0	0	0	0	0	0
19–26	0	0	0	0	0	0
27–28	1	1	0	1	0	0
29	1	0	0	1	0	0
Unknown	2	2	0	1	1	0
Total	46	35	9	32	11	3

ever, that none of the 14 patients with infections of less than 3 months' duration had spinal fluid infectious for the rabbit, while 4 out of 15 patients with infections of from 3 to 4 months' duration had infectious fluids

It is to be observed in Table III that in 2 patients with secondary manifestations of not over 2 weeks' duration, the spinal fluid was found to contain virulent organisms

Summary

The blood and cerebrospinal fluid of 46 Chinese patients in the active secondary stage of syphilis were inoculated into rabbits The blood proved to be infectious in 35, or 76 1 per cent, and the spinal fluid in 9, or 19 6 per cent, of the cases There was no correlation between a particular clinical lesion and infectivity of the spinal fluid, although the frequency of positive inoculations in patients with alopecia approached statistical significance.

No organisms were isolated from the spinal fluid of patients whose infections were less than 3 months' duration All patients with infectious spinal fluids also had virulent organisms in the blood

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CARDIOLIPIN AND ITS APPLICATIONS IN THE SERODIAGNOSIS OF SYPHILIS

By
*Mary C Pangborn**

The importance of serologic tests in syphilis has led to a vast amount of research on the improvement of such tests From the beginning, one of the difficulties in the way of such improvement has been the nature of the antigen

For many years the antigens most widely used have been variously prepared and modified alcoholic extracts of beef heart —crude solutions containing a very complex mixture of tissue constituents, chiefly lipides Two disadvantages are inherent in the use of such antigens—there is the difficulty of uniform standardization, since it is all but impossible to make two crude extracts exactly alike, and there is the question whether the nonspecific reactions encountered may be due to certain of the impurities in the antigens

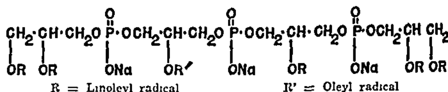
At the time when our laboratory took up this problem, many people had already attempted to purify the active principle of the extracts and it was known that the serologic activity was associated with the phospholipide fraction This in fact was the chief obstacle to purification—whenever active fractions were separated they turned out on examination to consist chiefly of lecithin

Our first approach was therefore to study the fractions separated during the purification of lecithin This led to the discovery of a highly important relationship of lecithin to the serologic activity Crude lecithin was serologically active, pure lecithin was inactive, but when the secondary fractions resulting from the purification were examined they also were found to be inactive or nearly so that is, if they were essentially lecithin free Only by recombining such fractions with the purified inactive lecithin could the serologic activity be demonstrated Thus even before the isolation of any new compound it was known that not one but two substances were required for serologic activity lecithin and some unidentified component The sensitizing effect of cholesterol already known to be an important factor in the case of crude extracts was demonstrated also with the separated phospholipide fractions The cardiolipin antigens in actual use

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are mixtures of three substances—cardiolipin, lecithin, and cholesterol

The "unknown" was finally isolated and proved to be a previously unrecognized phospholipide It is this new compound which has been named cardiolipin[1-4] The structure of the compound can be tentatively formulated as follows[5]:



(Positions of R and R', and of ester linkages, are arbitrary)

The structure is given as though only a single molecular species were present This, if true, would be rather surprising, since most lipides are mixtures of closely related homologues Recent evidence suggests that cardiolipin probably is a mixture of two closely similar compounds, one of which gives analytical values closely corresponding to the structure illustrated, while the other contains slightly less carbon The further characterization of these two fractions is an interesting chemical problem but fortunately it does not seem likely to introduce any practical complications, since no serologic differences have been detected between the two fractions

The compound differs from such phospholipides as lecithin and cephalin in that it contains no nitrogenous base, and also in the larger size of the structural unit Instead of glycerophosphoric acid, which is found in lecithin and cephalin, we have here a complex ester composed of at least three molecules of glycerophosphoric acid and one of glycerol The purified compound is used in the form of its Na salt, which is fairly soluble in alcohol The methods of isolation and purification depend on the formation of less soluble salts, such as those of Ba or Cd which can be purified by means of various combinations of organic solvents and finally reconverted to the desired Na salt for use in antigens

The particular advantages of the new antigen, as revealed by experience with it to date, are stability, reproductivity, ease of standardization and improvement of specificity.

Stability may be judged by the fact that antigen mixtures have been stored for periods up to eighteen months without any change in activity

Reproductivity and *ease of standardization* are no more than

should be expected as a result of purification, but these points need to be elaborated a little

One of the disadvantages of the use of crude tissue extracts has always been the difficulty in preparing uniformly reproducible antigens We should be able to eliminate this difficulty by the use of an antigen composed of essentially pure substances, provided that satisfactory standards of purity can be maintained for each of the ingredients For two of the three, this has presented no real difficulty It is relatively easy to prepare cardiolipin of uniform activity, and while the quality of commercially available cholesterol is variable, one can easily purify unsatisfactory lots by simple recrystallization from alcohol

Adequate purification of lecithin is more difficult New procedures were required for this purpose[4,6] and the experience of the past year has led to the adoption of still further improvements In our laboratory, we have been able to maintain a satisfactory constancy in the properties of lecithin made in the same way Undoubtedly this is the weakest link in the chain at present and more study of improved methods for lecithin purification is needed This problem is not serious in the case of the antigen for the complement-fixation test, but antigens for precipitation methods are significantly affected by slight differences in the purity of different lots of lecithin

Another point requiring further study is the possibility of using lecithins from sources other than beef heart Not enough work has yet been done to show to what extent adequately purified lecithins from different sources might be interchangeable in antigens

While still on the subject of standards of purity, we should perhaps speak of four rather than three antigen ingredients since a recent unhappy experience has emphasized the need of securing a good quality of alcohol for the antigen solutions We have found that some of the absolute alcohol now on the market is not suitable for use in antigens without redistillation

The standardization of each new lot of crude extract antigens has always required an elaborate series of serologic tests With a purified antigen, it seems clear that this should no longer be necessary Once the proportions of the three components required for a given serologic test have been determined, each new lot of cardiolipin or lecithin may be tested by making up an antigen of the predetermined composition and using it in tests of a number of positive and negative sera in parallel with a pre-

viously used standard antigen If the reproductivity of the purification methods is satisfactory, obviously different lots should give the same result *when tested in the same concentration*, if they do not, the serologic test has provided evidence that the separately prepared lots are not identical In our laboratory we regard such disagreements as indication of unsatisfactory purification It is granted that as methods are improved, it may be necessary from time to time to revise the conditions of a particular test procedure, but if we are going to try to compensate for inadequate purification by changing the formula of each batch of antigen and going through a complete serologic restandardization of each lot, we are simply discarding one of the important advantages of a purified antigen

Before discussing sensitivity or specificity, it is necessary first to ask how the new antigen can actually be adapted to serodiagnostic use The problem is complex because we are dealing not with a single substance but with a delicately balanced colloidal mixture of three components The relationships of these three components—cardiolipin, lecithin, and cholesterol—are therefore of basic importance The first and most thorough studies of this question were those carried out by Doctor and Mrs Maltaner on the standardization of the antigen for use in the quantitative complement-fixation test developed by them[7], and by Dr Rachel Brown on a tube precipitation test[8]

Cardiolipin and lecithin were first tested separately Neither substance alone had any significant activity in either test Mixtures of cardiolipin + cholesterol or of lecithin + cholesterol had no activity in either test Mixtures of cardiolipin and lecithin, however, were active antigens in both types of tests As yet there is no explanation for this combined effect of the two phosphatides

The method of study was, therefore, first to determine the optimum proportions of cardiolipin and lecithin, and then to study the reaction with varying amounts of added cholesterol Certain relationships proved to be common to both serologic procedures In both cases a fairly definite optimum ratio of lecithin to cardiolipin was found, and this ratio was not altered by the addition of cholesterol The effect of cholesterol was to increase the sensitivity of the antigen mixtures

In the last few years several authors have published descriptions of their experience with the adaptation of cardiolipin and lecithin to other test procedures In general, the various serologic test procedures require different proportions of the three

components and each therefore presents a separate problem of study From all these reports to date, it seems clear that cardiolipin antigen can be successfully applied in any test for which beef heart antigens have been used The relative value of the various tests proposed will have to be determined by extensive further trial In any such evaluation the questions of the sensitivity and specificity of the new antigen must be considered

Whatever the test procedure, it can hardly be over emphasized that failure to make use of a properly balanced mixture will result in an unsatisfactory antigen In fact, it is possible so to oversensitize an antigen mixture that false positive readings may result For instance, in the early studies of the complement-fixation test, an antigen was tested which contained a smaller proportion of lecithin to cardiolipin than the mixture finally chosen for use The ratio of lecithin to cardiolipin in this mixture was 1 43 1, whereas in the mixture now used, it is 5 1 The antigen with the ratio of 1 43 1 had been selected in preliminary tests because it was more reactive On trial in the routine service, however, it gave occasional reactions that were considered nonspecific and was therefore rejected The later experimental studies also indicated that such an adjustment would be unsatisfactory

In certain cases there is already clear evidence that purification of the antigen has increased its specificity Thus the early work in the Division of Laboratories and Research of the New York State Department of Health[9] indicated that the cardiolipin antigen would prove somewhat more specific in patients with malaria and in vaccinated persons, cases in which false positive results are often reported Recent work has confirmed this Similar conclusions were reported by Rein and Bossak[10], Rein and Kent[11], and by Kline[12], who found cardiolipin antigens much more specific than crude antigens in cases of malaria Equally convincing data on other types of false positive reactions are not yet available, but all the evidence so far indicates that the specificity of the purified antigen is definitely greater But here it should be emphasized that the removal of impurities is undoubtedly only one factor in the observed improvement Another important point is that when the purified ingredients are used the proportion of the three can be adjusted In a crude extract the proportion of cardiolipin, lecithin and cholesterol cannot be accurately determined and some nonspecific reactions observed with crude extracts may well have been due to variations in these proportions

However, a word of caution is needed on this question of specificity In the excitement of having reasonably pure materials available for compounding antigens, too much emphasis has perhaps been placed on the results to be expected from purification and not enough on the limitations and peculiarities of this particular antigen system Thus, while we could predict with confidence that purification would give us a more uniform and easily standardized antigen, it could only be hoped, not predicted, that any improvement in specificity would result That such improvement has resulted in certain cases is a great advantage but there seems to be no basis for assuming that all "false positive" reactions are related to properties of the antigen Many such cases will doubtless have to be approached from the other side, by a study of the serum, as has been done by Neurath and his associates[13]

But even when the antigen is the decisive factor, we have seen that the mere possession of the purified substances is not enough The antigen is a complex colloidal system very sensitive not only to variations in the proportion of the components but also to such technical factors as methods of dilution and heating of antigen suspensions By variations in such details both sensitivity and specificity may be altered, and the very fact of this flexibility in adjustment raises new problems which lay a heavy responsibility on the serologist

It is evidently quite feasible to adjust the activity of cardiolipin antigens so that the results will be comparable in sensitivity to those obtained with extract antigens This, however, is only a partial solution Until now, as each test has been studied, the sensitivity has usually been set by making the performance of the new antigen match that of some previously accepted test This is obviously a necessary beginning For the future, however it would seem that a thorough reappraisal of present practices is in order Collaboration between clinicians and serologists will be essential to determine the level of sensitivity to which serologic tests with the new antigen should be adjusted It is to be hoped also that some agreement on standardization of serologic practices can be reached so that results obtained in different laboratories can at least be evaluated in common terms The availability of a purified antigen should be an occasion for continued progress in this direction

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CARDIOLIPIN-LECITHIN-CHOLESTEROL ANTIGEN IN THE KAHN TEST III*

By

Reuben L Kahn†

It is a pleasure to appear on this program with Dr. Pangborn Her emphasis that the purified lecithin problem is not yet solved fits in with our findings that different lots of purified lecithin give different serologic results In this brief discussion, several aspects of cardiolipin antigen in its behavior with salt solution and with serum will be considered in comparison with Kahn antigen

Variations in Sensitivity of Different Lots of Cardiolipin Antigen, Apparently Due to Differences in Lecithin

One of the earliest observations made in this laboratory in the use of cardiolipin antigen in the Kahn test was that different lots may show different sensitivities, although prepared according to the same formula The question arose whether these differences in sensitivity were due to differences in lecithin or to differences in cardiolipin Experiments showed that three different lots of cardiolipin employed with one lot of lecithin gave practically identical sensitivity results When, however, one lot of cardiolipin was employed with two different lots of lecithin variations in results were obtained Table 1 illustrates these variations with cardiolipin antigens prepared with two lots of purified lecithin It is evident that cardiolipin antigen prepared with lecithin 'Run 1' gives considerably more sensitive results than the antigen prepared with lecithin 1A-46 This and similar results obtained with other lots of purified lecithin, pointed to the necessity of standardizing each lot of cardiolipin antigen before its use in the Kahn test

The Cardiolipin Antigen Formula for Use in the Kahn Test

The formula consists of 1 0% purified lecithin, 0 1% cardiolipin and 0 025% cholesterol When employing this formula, the resulting antigen will produce suspensions with salt solution

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TABLE I

Illustrating Differences in Sensitivity of Cardiolipin (P) Antigen With Two Different Lots of Purified Lecithin

Serum No	Standard Kahn Reactions with Cardiolipin Antigen Employing											
	Lecithin 1A-46						Lecithin Run 4					
	First Reading*			Second Reading			First Reading			Second Reading		
	Tubes 1	2	3	Tubes 1	2	3	Tubes 1	2	3	Tubes 1	2	3
1	—	1	3	—	—	2	—	3	4	—	3	4
2	—	1	3	—	—	—	—	2	4	—	2	4
3	—	—	3	—	—	2	—	3	3	—	2	2
4	—	±	2	—	±	2	3	4	4	3	4	4
5	—	3	4	—	2	4	—	4	4	—	4	4
6	—	1	3	—	2	2	—	3	4	—	2	3
7	—	4	4	—	4	4	3	4	4	3	4	4
8	—	—		—	—	—	—	1	1	—	1	1
9	2	3	4	2	3	4	3	4	4	3	4	4
10	—		—	—	—	—	—	—	—	—	—	—
Total Plus signs	41			33			69			65		

* The first reading was made after the addition of diluent the second reading was made 15 minutes later

similar to suspensions given by Kahn antigen and it will behave with syphilitic and nonsyphilitic serums similar to the behavior of Kahn antigen

Of interest is the fact that when employing the same ratios of lecithin to cardiolipin namely 10 1, but of different concentrations of these reagents workable antigens are not obtained For example 0 5% of lecithin 0 05% of cardiolipin and 0 025% of cholesterol do not lead to a workable antigen The same holds true if 2 0% lecithin 0 2% cardiolipin and 0 025% cholesterol are employed Evidently the 10 1 ratio of lecithin to cardiolipin combined with the appropriate concentration of these reagents is of importance in obtaining a workable cardiolipin antigen for the Kahn test Table 2 illustrates these findings

As is noted from the table the antigen titer is 1 ml antigen plus 0 9 ml salt solution (0 9%) If 1 ml antigen is mixed with 0 8 ml salt solution the resulting antigen suspension gives cloudy mixtures with serum and it is impossible to differentiate syphilitic from nonsyphilitic serums If 1 ml antigen is mixed with 1 ml of salt solution the resulting antigen suspension gives

TABLE 2

Concentrations of Lecithin and Cardiolipin (10 1 Ratio) Producing an Antigen Suspension Usable in the Kahn Test

Antigen + Salt Solution ml	Cardiolipin Antigen Formula A	B	C
	0 5% Lecithin 0 05% Cardiolipin 0 025% Cholesterol	1 0% Lecithin 0 1% Cardiolipin 0 025% Cholesterol	2 0% Lecithin 0 2% Cardiolipin 0 025% Cholesterol
	Total Lipid Concentration		
	0 575%	1 125%	2 225%
1+0 8	Too clear Some Nondispersible aggregates	Cloudy Nondispersible aggregates	Turbid Nondispersible aggregates
1+0 9	Nearly water clear	Opalescent*	Turbid Nondispersible aggregates
1+1 0	Water clear	Too clear	Cloudy Nondispersible aggregates
1+1 1	Water clear	Much too clear	Cloudy Some nondispersible aggregates
1+1 2	Water clear	Nearly water clear	Opalescent Some nondispersible aggregates

* A usable antigen suspension was obtained with Formula B when employing 1 cc antigen + 0 9ml salt solution Lecithin Lot 12A and Cardiolipin Lot 13 employed in this experiment

altogether too clear mixtures with serum and the suspension of markedly reduced sensitivity. Briefly, the antigen must b mixed with salt solution at the titer, namely, 1 + 0 9, to obta a usable antigen suspension in which syphilitic serums will sho precipitates and nonsyphilitic serums will show the opalescen characteristic of negative reactions with Kahn antigen

The narrow titration range of cardiolipin antigen is of in terest when compared to Kahn antigen As is well known, th latter antigen has a relatively wide titration range For exampl if Kahn antigen has a titer let us say of 1 + 1 3, then antige suspensions prepared by adding 1 2 ml of salt solution to 1 m of antigen or 1 4 ml to 1 ml of antigen are likely to give result *closely similar to that obtained when the antigen is mixed a* the titer of 1 + 1 3 Briefly, 0 1 ml of salt solution above o below the titration point apparently does not ...
the physical ...
case ...
or be ...
suspe ... between Kahn and cardiolipin anti-

gen is based on the probability that Kahn antigen contains nonantigenic colloids which are protective in nature, while cardiolipin antigen, because of its high purity, lacks these protective colloids Someone has compared the two antigens to buffered and unbuffered solutions

Other ratios of lecithin and cardiolipin were tried also with different concentrations of lecithin and cardiolipin but the most clear cut titration point was obtained in a 10 1 ratio of lecithin to cardiolipin in which 1 0% lecithin and 0 1% cardiolipin were used In the case of the cholesterol, of the various concentrations tried, 0 025% gave workable results

It is believed that cardiolipin antigens which are undersensitive may be raised in sensitivity by small increases in the lecithin cardiolipin ratio Thus, in those instances in which a 10 1 ratio of lecithin-cardiolipin gives undersensitive results, a 11 1 ratio will show increased sensitivity A 12 1 ratio will increase the sensitivity still further, but in that ratio weak nonspecific reactions begin to appear Similarly when a cardiolipin antigen is oversensitive, sensitivity can be lowered by reducing the lecithin cardiolipin ratio Thus, a 9 1 lecithin-cardiolipin ratio will give somewhat lower sensitivity results than a 10 1 ratio An 8 1 ratio will give results of still lower sensitivity

Comparative Kahn Results with Cardiolipin and Kahn Antigen

The results herewith presented consist of cardiolipin antigen prepared with the formula indicated above, namely 1 0% lecithin, 0 1% cardiolipin and 0 025% cholesterol The titer of this antigen employed, as already indicated, was 1 + 0 9 When employing cardiolipin antigen at this titer with syphilitic and nonsyphilitic serums, the first observation that one notes is that the general precipitation picture in the 3 tubes of the Kahn test are the same with both antigens Considering the high purity of cardiolipin antigen and the fact that Kahn antigen contains considerable amounts of nonantigenic lipids, it is truly remarkable that the two antigens should show such similarity in results

Table 3 gives comparative Kahn reactions with cardiolipin and Kahn antigens in 1,910 routine serologic examinations As indicated in the table, 163 serums gave positive Kahn reactions and 1,713 serums gave negative reactions with both antigens Nine serums giving positive reactions with Kahn antigen, gave negative reactions with cardiolipin antigen, while two serums

TABLE 3

Kahn Reactions with Cardiolipin Antigen Compares with Kahn Reactions with Kahn Antigen in 1910 Routine Examinations

Number of Sera	Standard Kahn Reactions with Kahn Antigen	Standard Kahn Reactions with Cardiolipin Antigen	Analysis of Results Number	Per Cent
			Agreement	
163	Positive	Positive		
0	Doubtful	Doubtful		
1713	Negative	Negative	1876	98 2
			Relative Agreement	
4	Positive	Doubtful		
1	Doubtful	Positive	5	0 3
			Disagreement	
9	Positive	Negative		
12	Doubtful	Negative		
2	Negative	Positive		
6	Negative	Doubtful	29	1 5

giving positive reactions with cardiolipin antigen, gave negative reactions with Kahn antigen The results with Kahn antigen are thus somewhat more sensitive than the results with cardiolipin antigen

A technical step has recently been observed in this laboratory which renders the sensitivity of cardiolipin antigen more comparable to that of Kahn antigen This technical step consists of centrifugation of the completed cardiolipin tests for 15 minutes at about 2,000 r p m Centrifugation causes the minute particles in negative reacting serums to go to the bottom of the tube On tilting the tube, these particles tend to rise from the bottom in the form of a whirl, without any tendency toward clumping In the case of positively reacting serums, centrifugation tends to clump the floccules and clear the medium

Centrifugation of Kahn tests with cardiolipin antigen has been applied in this laboratory to more than 1,000 negative Kahn reacting serums with Kahn antigen It was found that seven of these serums gave weakly positive reactions with cardiolipin antigen These serums gave positive Kahn presumptive reactions and there is reason to believe that the results were specific The use of centrifugation in borderline or negative cardiolipin reactions in the face of positive Kahn reactions, it is believed, will lift up the sensitivity of those reactions close to the level of Kahn antigen

Microflocculation Reactions with Cardiolipin Antigen Compared with Kahn Reactions with Kahn Antigen

When turning to microflocculation reactions with cardiolipin ntigen the comparison with standard Kahn reactions is much

TABLE 4

Standard Kahn Reactions Compared with Microflocculation Reactions with Cardiolipin Antigen in 2034 Routine Examinations

Number of Sera	Standard Kahn Reactions with Kahn Antigen	Microflocculation Reactions with Cardiolipin Antigen	Analysis of Results Number	Per Cent
			Agreement	
161	Positive	Positive		
10	Doubtful	Doubtful		
821	Negative	Negative	1992	97 9
			Relative Agreement	
12	Positive	Doubtful		
5	Doubtful	Positive	6	0 3
			Disagreement	
2	Positive	Negative		
1	Doubtful	Negative		
5	Negative	Positive		
28	Negative *	Doubtful	36	1 8

* Kahn presumptive test positive or doubtful with sixteen sera

closer Table 4 gives the standard Kahn results compared with microflocculation reactions with cardiolipin antigen in 2,034 routine serologic examinations As indicated in the table, 161 serums gave positive reactions and 1 821 serums gave negative reactions with Kahn and cardiolipin antigens Twelve serums giving positive reactions with Kahn antigen gave doubtful reactions with cardiolipin antigen Two serums giving positive reactions with Kahn antigen gave negative reactions wth cardiolipin antigen while five serums giving negative reactions with Kahn antigen gave positive reactions with cardiolipin antigen It is evident from the results in the table that the sensitivity of the microscopic procedure with cardiolipin antigen closely parallels the sensitivity of Kahn reactions with Kahn antigen

Microflocculation Reactions More Sensitive than Test Tube Reactions with Cardiolipin Antigen

Microflocculation tests are the more sensitive because no diluent is added to the serum antigen mixtures before reading the results The diluent consisting of 0 3, 0 1 and 0 1 ml of 1 2%

NaCl solution, added respectively, to the three tube cardiolipi tests, tends to disperse certain precipitates Hence the fin results of the tube tests are in some instances weaker than th results of the slide tests

Of interest is the fact that if the micro amounts of seru and antigen suspension used in the slide tests are employed i tube tests and shaken for three minutes in the Kahn shaker, th results of these tests when read without diluent are more sensi tive than the results of the slide tests The reason for this in creased sensitivity, it is believed, lies in the stronger agitation of the tube tests in the shaking machine than of the slide tests The serum antigen suspension mixture on slides cannot be agi tated as forcefully as in test tubes The sensitivity of these micro tube tests without diluent is indeed so marked as to show a tendency toward nonspecificity

Single versus Multiple Ratios of Serum—Antigen Suspension in a Test for Syphilis

It is well to keep in mind the limitations of single serum-antigen suspension ratios in tests for syphilis Many years ago when I developed the standard Kahn test, I felt that three ratios of serum antigen suspension represented the smallest number of ratios that should constitute a dependable test The reason for this belief was the fact that different syphilitic serums show optimal reactivity not in one precipitation zone of a given serum antigen ratio but in different precipitation zones of different serum antigen ratios Table 5 illustrates these different precipi tation zones

It is evident from the table that serums 1 and 2 show optimal reactivity in precipitation zone III where there is a marked increase of serum over antigen suspension Serums 3 and 4 show optimal precipitation where there is an increase of antigen sus pension over serum and serums 5 and 6 show optimal precipita tion with a moderate increase of serum over antigen suspension It is believed that individual cases of syphilis, especially when untreated, can best be detected by employing a test comprised of at least three ratios of serum antigen suspension Such a test affords a means for the better understanding of the reactions of individual cases In addition, the use of three tubes with three different ratios of serum antigen suspension obviously affords a more reliable technical means in the detection of syphilis than the use of a single ratio of serum antigen suspen

TABLE 5

Illustrations of Optimal Precipitation Zones Shown by Different Syphilitic Serums

Serum No	Serum Kahn Antigen Suspension Zone I			Zone II (Standard Kahn)			Zone III			
	1 2	1 1	2 1	3 1	6 1	12 1	24 1	48 1	76 1	100 1
	Optimal Precipitation with Increase of Serum over Antigen									
1	—	—	—	2	4	4	4	4	4	4
2	—	—	—	—	—	3	4	4	4	4
	Optimal Precipitation with Increase of Antigen over Serum									
3	4	4	4	4	4	2	—	—	—	—
4	4	4	4	3	—	—	—	—	—	—
	Optimal Precipitation with Moderate Amounts of Serum and Antigen									
5	—	—	2	4	4	4	4	2	—	—
6	—	—	—	—	2	4	3	1	—	—
	Precipitation in all Ratios									
7	4	4	4	4	4	4	4	4	4	4
	No Precipitation in any Ratios									
8	—	—	—	—	—	—	—	—	—	—

sion It is true that in statistical comparisons of tests, the value of the use of three ratios of serum-antigen suspension may not be apparent In the study of individual persons with syphilis, however, the value has been proved by 25 years of experience with the Kahn test

TECHNIQUES

I Cardiolipin Antigen in the Standard Kahn Test with Serum

The techniques with cardiolipin antigen herewith outlined are, in the present state of our limited knowledge of the clinical value of this antigen, recommended for use side by side with the standard Kahn test employing Kahn antigen

1 Performance of Test

1) Arrange test tubes in standard Kahn racks so that there are three tubes for each serum to be tested, including positive serum, negative serum and saline controls Number the first row of tubes to correspond to the serums being tested, using a different colored wax pencil than that used in numbering tubes for tests with Kahn standard antigen

2) Prepare cardiolipin antigen suspension as follows:

a Measure into an antigen suspension vial the amount of

saline (0 9% NaCl solution) according to titer, re quired for the given amount of antigen

Note—The titer on the bottle will state the amount o saline that must be mixed with 1 ml of antigen n order to prepare a proper suspension Less tha 1 ml or more than 2 ml of antigen should not be measured in one mixing vial

b Measure into a second antigen suspension vial the necessary quantity of antigen

c Pour the saline into the antigen and, without stopping pour the mixture back and forth 12 times, without allowing vials to drain during mixing period

d Allow the antigen suspension to age 10 minutes before using Do not use suspension after it has aged more than 30 minutes from the time of mixing

3) Before pipetting the antigen suspension, place thumb over mouth of vial and shake gently to suspend antigen par ticles

4) Pipette 0 05 ml of the antigen suspension directly to the bottom of each tube of the first row of the Kahn rack, employing a Kahn antigen pipette

5) Pipette 0 025 ml of antigen suspension directly to the bottom of each tube of the middle row of the Kahn rack, employing a Kahn antigen pipette

6) Pipette 0 0125 ml of antigen suspension directly to the bottom of each tube of the back row of the Kahn rack, employing a Kahn antigen pipette

7) Add 0 15 ml of each serum to the designated set of three tubes containing 0 05 ml, 0 025 ml and 0 0125 ml antigen suspension, respectively

Note—The heating of the serum for 30 minutes at 56° C the absolute clarity of the serum and freedom from particles and the set up of the 3 tube test are the same as in the Kahn test with standard antigen The serums should be employed soon (if possible, within 10 min utes) after the heating period

8) Shake rack by hand for 10 seconds, after antigen sus pension and serum have been added to all tubes in that rack

9) Permit serum antigen suspension mixture to stand for 3 to 7 minutes at room temperature

10) Shake rack of tubes for 3 minutes in Kahn shaking ma chine (275 to 285 oscillations per minute)

11) Following the 3 minute shaking period, add 0 3, 0 1 and 0 1 ml of 1 2% solution to tubes 1, 2 and 3, respectively
    Note—In this respect the Kahn test with cardiolipin antigen differs from the Kahn test with Kahn antigen, in which 1 0, 0 5 and 0 5 ml of 0 9% salt solution are added to tubes 1, 2 and 3, respectively

2 Reading of Results
  1) Read each tube of the rack immediately after adding the 1 2% salt solution and agitating rack gently to mix the ingredients (Make only one reading)

3 Methods of Reading of Results of Kahn Reactions with Cardiolipin Antigen

  Note—The reading of results of Kahn reactions with cardiolipin antigen is based on the same criteria as the reading of results of Kahn reactions with Kahn antigen The precipitates in the positive reactions with cardiolipin antigen are not quite as heavy as those with Kahn antigen, but sufficiently heavy to be readily differentiated as 4+, 3+, 2+, 1+ and ± The negative reactions with cardiolipin antigen when highly magnified, will show the presence of finely dispersed particles (very likely antigen particles) to a somewhat greater degree than the negative reactions with Kahn antigen However, under proper routine conditions of reading results with either antigen, no difficulty will be encountered in differentiating the negatives even from the doubtful reactions, once the reader becomes familiar with the appearance of the negatives

  No standardized method for reading the results could be made applicable to all workers because 1) different workers have different visual capabilities and 2) different laboratories have different reading facilities

  1) *Reading by Window Light* When reading Kahn reactions with cardiolipin antigen by holding the rack of tubes in front of a window in a dimmed room, the negatives will appear opalescent and clear, and the positive reactions will appear turbid or cloudy The four plus reactions showing heavy precipitation can be readily observed without lifting the tubes from the rack All other tubes showing any degree of turbidity or clouding should be examined individually, lifting each tube several inches above the eye level and slanting it until the fluid is spread into a thin layer The precipitate will then become readily visible It is important 1) For the reader to stand close

to the window, which provides the source of light for reading, 2) For the other lights in the room to be dimmed thereby preventing other sources of light from playing on the tube, 3) For the slanted tube to be held several inches *above* the eye level Ideally, the window used for reading should be shaded in its upper and lower parts in such a way as to leave a trip of light, the width of which to be determined for each laboratory, for optimal reading of results

2) *Reading by Slit-lamp or by Fluorescent Light* Those reading by artificial light will find it desirable by so holding the rack in front of the light as to readily differentiate the opalesecent and clear negatives from the cloudy and turbid positives Then, the positive reactions are examined individually Tubes showing borderline clouding should, of course, also be examined individually As in reading by window light, it is important to dim other lights in the vicinity of the reader

3) *Reading by Magnification* When employing the concave surface of the microscope mirror for reading results, it is also well to first differentiate the negative reactions from those showing some degree of turbidity, as explained above Those who find the magnification of the microscope mirror insufficient may combine the mirror with a hand lens *The magnification must be sufficiently low for an individual reader to assure that the negative reactions appear opalescent and easily distinguishable, when the negative reactions begin to suggest the appearance of doubtful reactions, then the magnification is too high for that reader* As is well known, any colloidal solution, sufficiently magnified, will show particles

4) *Qualifications for Reading Kahn Reactions with Cardiolipin Antigen* It is assumed that those who will read Kahn reactions with cardiolipin antigen will have had ample experience in reading Kahn reactions with Kahn antigen Workers without this experience should first learn to read Kahn reactions with Kahn antigen

4 Employment of Centrifugation

Centrifugate for 15 minutes at about 2,000 r p m, tests with cardiolipin antigen that give borderline or negative reactions in the face of positive reactions with the Kahn test with Kahn antigen The minute particles in the negative reactions tend to go to the bottom of the tubes On tilting

the tube these particles rise from the bottom in the form of a whirl without any tendency toward clumping In the case of positively reacting serums centrifugation tends to clump the floccules and clear the medium

Note—The use of centrifugation in the instances described above it is believed will lift up the sensitivity of the reactions close to the sensitivity level of Kahn reactions with Kahn antigen

Reporting of Results

1) Average the results of the Kahn test with cardiolipin antigen in the same manner as in the Kahn test with Kahn antigen

II Cardiolipin Antigen in the Kahn Microflocculation Test with Serum

1 Performance of Test

1) Employ in the microscopic procedure the same cardio lipin antigen suspension described above used in the 3 tube test
2) Let the cardiolipin antigen suspension age 10 minutes before using and discard it after it has aged for more than 30 minutes
3) Assure absolute clarity of the serum and freedom from particles as well as clean slides
4) Deposit on a paraffin ringed slide 0 05 ml amounts of the serum previously heated for 30 minutes at 56° C
5) Mix the antigen suspension well by drawing it up several times in a tuberculin syringe with a 23 gauge needle Hold syringe vertically over the serum and permit a drop of the suspension to fall in the center

   Note—The serum antigen suspension ratio is then approximately 8 1
6) Vigorously agitate the slide in a circular motion (150 160 times per minute) for 4 minutes and read the results without delay

2 Reading of Results

1) The results are read under low power magnificat on of approximately X 50

   a *Negative reactions* show in the microscopic field evenly distributed very small non aggregated particles

   b *Doubtful* (±) *reactions* show a microscopic field crowded with numerous small aggregates or clumps

   c *Doubtful* (+) *reactions* show slightly larger aggre gates somewhat less crowded

d *Positive (++) reactions* show larger aggregates with a corresponding clearing of the field

e *Positive (+++) reactions* show relatively large aggregates scattered through the clear field

f *Positive (++++) reactions* show but few large clumps in a clear field

KAHN REACTIONS WITH KAHN AND CARDIOLIPIN ANTIGEN—DISCUSSION

In this laboratory cardiolipin antigen is used side by side with Kahn antigen in the Kahn test It is believed that the two *antigens, because of their differences in lipid content, make a* good team in the serodiagnosis of syphilis In the presence of syphilis, there is reason to believe that the two antigens will have selective reactivities, one antigen detecting particular cases of syphilis that the other might miss In the absence of syphilis cardiolipin antigen is definitely more specific in malaria and it may prove to be also more specific in other conditions

Thus far, the standard Kahn test with Kahn antigen has shown a unique record of specificity in all official competitive evaluation studies since 1928 During the past decade, the test has given 100 per cent specificity results in all evaluation studies extending from 1937 to 1947, inclusive The number of serums examined in these evaluations are 2,293 About 900 of the serums came from donors with various pathologic conditions excluding leprosy and malaria The evaluation results are summarized in Table 6 It is clear that tests with cardiolipin anti

TABLE 6

Thirteen Consecutive Official Evaluation Studies (1937—1947) in which the Kahn Standard Test gave No False Positive or Doubtful Reactions

Evaluation Study Year	Number of Non syphilitic Cases	Specificity Per Cent	Clinical Condition of Donors
1937	100	100	Normal
1938–1	96	100	Normal
1938–2	444	100	Tuberculosis
1939	114	100	Normal
1940	111	100	Normal
1941 1	130	100	Normal
1941 2	453	100	Malignancy fever etc
1942	129	100	Normal
1943	131	100	Normal
1944	161	100	Normal
1945	153	100	Normal
1946	135	100	Normal
1947	136	100	Normal

gen have yet to establish such a specificity record Furthermore, the specificity record of those tests must conform to a given sensitivity range—a range not below that of the standard Kahn

There is but one way to determine the superiority of a given test with cardiolipin antigen, namely, by official competitive evaluation studies To illustrate Suppose author A of a test is assigned to a given laboratory room and, in the course of a week or ten days, is called upon to examine, let us say, 1,000 "unknown" specimens of blood from selected clinical material He examines these specimens with his particular test, without benefit or detriment of supporting data from batteries of tests He turns in his results in sealed envelopes These results are then compared by appropriate authorities with the clinical findings and with serologic results submitted by author B who performed his particular test, author C, his test, etc It is obvious that under such conditions, a test is truly evaluated

Without official evaluation studies, the value of a method must remain a matter of opinion The most well intentioned worker, fully believing in his particular method based on given data, may be altogether mistaken as to the value and limitations of the method Without evaluation studies, we shall soon find that the number of tests proposed will become well nigh innumerable, each author in all honesty claiming superiority of his particular method Even the limited annual evaluation of serologic laboratories of State Health Departments are of importance in throwing light on the value of a test For example, I would not have believed that the sensitivity of the Kahn test might be higher than the sensitivity of the New York State complement fixation and flocculation tests with cardiolipin antigen But in the 1947 evaluation of laboratories carried out by Doctor Mahoney, the New York State flocculation test gave 75 0% sensitivity, the complement fixation test gave 73 3% sensitivity and the standard Kahn test with Kahn antigen gave 81 6% sensitivity

The greatest value of official evaluation studies lies in the fact that they throw light on the value and the limitations of a method impartially and without personal bias

Summary

1 Cardiolipin lecithin-cholesterol antigen in the Kahn test must be standardized serologically, lot by lot, mainly because of serologic variations in the purified lecithin

2 The cardiolipin antigen formula, adaptable to the Kahn

test, consists of 1 0% purified lecithin, 0 1% cardiolipin and 0 025% cholesterol It is believed that small changes in this formula should satisfy standardization needs, when faced with variations in the reagents

3 Kahn results with cardiolipin antigen approach the sensitivity of results with Kahn antigen, especially if centrifugation is applied to borderline reactions with the cardiolipin tests

4 The micro slide cardiolipin test, herewith described is somewhat more sensitive than the standard Kahn 3 tube test with the same antigen This increased sensitivity is due to the fact that no diluent is added to the slide test, while the diluent added to the tube test tends to disperse some precipitates

5 It is believed that multiple ratios of serum antigen suspension in a test for syphilis give, in the long run, more dependable results in individual cases of syphilis than does a single ratio of serum antigen suspension

6 The determination of the value and limitations of a test with cardiolipin antigen, as in the case of any new serologic test for syphilis, can be made only by official and impartial evaluation studies in which the proposed test is employed in the examination of "unknown" specimens

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PRELIMINARY EVALUATION OF A COOPERATIVE STUDY ON THE EUGLOBULIN-INHIBITION TEST FOR THE SEROLOGIC DIFFERENTIATION BETWEEN TRUE AND BIOLOGIC FALSE POSITIVE REACTIONS FOR SYPHILIS

Hans Neurath†

Under the auspices of the Syphilis Study Section of the Division of Research Grants and Fellowships, National Institute of Health, U S Public Health Service, a cooperative survey study was organized in 1946, with the object of evaluating the euglobulin inhibition test[1] when carried out in hands other than those of the originators of this technic The results which are presented herein are preliminary to a full report which will be made after completion of the survey study*.

The details of the principles of the euglobulin inhibition test have been recently described in a series of publications[1-5] The essential features of this method are as follows

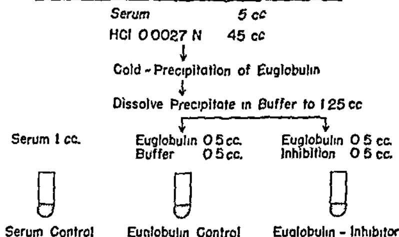
1 A serum protein fraction is isolated from whole serum by a process of isoelectric precipitation under controlled conditions of protein concentration, pH and temperature This fraction, which according to its solubility characteristics is denoted as "euglobulin," contains 50 per cent or more of the total serologic activity of the whole serum It contains about 7 per cent of the total serum proteins, 13 per cent of the *gamma* globulin fraction, and is essentially free of serum albumin

Quantitative serologic titrations are carried out on this euglobulin fraction, using a phosphate-saline buffer, pH 6 8 as diluent

2 Another serum protein fraction, which is currently obtained as a by product of the plasma fractionation program of the American Red Cross, Fraction IV-1, contains an inhibitor which specifically inhibits the flocculation reaction of euglobulin fractions of human sera from individuals who give biologic false positive reactions for syphilis This serum protein fraction contains mainly *alpha* globulins, associated with about 15 per cent lipoidal material of which about 7 per cent comprises cholesterol

* Since it has not yet been [illegible]
sults to a cooperative [illegible]
ideas expressed [illegible]

and cholesterol esters[6] In order to demonstrate inhibition, or lack of inhibition, another aliquot of the euglobulin fraction is subjected to quantitative serologic titrations in the presence of this inhibitor, serial two fold dilutions being made with specified concentratons of the inhibitor as diluent A graphical illustration of the method and of the types of results that can be obtained is given in Fig 1



Since experience has shown that serologic titrations with cardiolipin antigens yield fewer equivocal results than serologic titrations with the cruder beef heart antigens, the present evaluation was limited to analyses obtained with two cardiolipin antigen emulsions These are 1 The Rein Bossak cardiolipin emulsion (RB) and 2 the cardiolipin antigen emulsion developed at the Venereal Disease Research Laboratory (VDRL) Typical results obtained with a syphilitic and with a biologic false positive human serum are illustrated in Table I

The laboratories who have participated in this survey were a) The Division of Serology, Army Medical School, Washington

TABLE I

Representative Example of a Routine Euglobulin Inhibition Test

Fraction	Conc	Dilution				
		Undil	1 2	1 4	1 8	1 16
Syphilitic Type						
Serum	1x	3	2	1	0	0
Euglobulin	2x	3	2	1	0	0
Euglobulin + Inhibitor	2x	3	1	±	0	0
Biologic False Positive Type						
Serum	1x	2	1	±	0	0
Euglobulin	2x	4	4	4	2	0
Euglobulin + Inhibitor	2x	0	0	0	0	0

D C**, b) the Venereal Disease Research Laboratory, Staten Island, N Y†, c) the laboratories of Dr Charles R Rein, New York, N Y‡, and d) the laboratories of Duke University School of Medicine, Durham, N C

The serologic technics of these four laboratories were standardized by the exchange of serum specimens and by mutual consultations of the technicians The serum specimens used in the survey were initially numbered by code and the results obtained in each laboratory submitted to Duke University for evaluation Clinical histories of the patients from whom serum specimens were obtained were subsequently submitted, the sera decoded and the serologic results compared to the clinical histories Only those sera which clinically were definitely of the syphilitic or biologic false positive type were considered in this survey No consideration was given to the initial serum titer, to the stage of the disease or to the extent of antisyphilitic therapy that was administered to syphilitic patients, nor was the clinical cause for a biologic false positive reaction regarded as a limiting factor in the selection of sera The single exception was sera from

** Mr John F Kent has been in charge of the work carried out in that laboratory

† Mr Ad Harris has been in charge of the work carried out in that laboratory

‡ Dr Charles R Rein has been in charge of the work in that laboratory

TABLE II

Known Biologic False Positive Human Sera

Serologic Type	Antigen	Laboratory							
		I		II		III		IV	
		No	%	No	%	No	%	No	%
B T	RB	38	22 3	24	17 9	41	24 4	30	21 1
	VDRL	36	21 2	29	21 7	49	29 2	29	20 4
B T ?	RB	11	6 4	9	6 6	6	3 6	11	7 7
	VDRL	7	4 2	6	4 4	7	4 2	10	7 0
S T	RB	6	3 5	5	3 7	0	0	3	2 1
	VDRL	2	1 2	0	0	0	0	2	1 4
S T ?	RB	1	0 6	2	1 5	0	0	1	0 7
	VDRL	2	1 2	0	0	0	0	1	0 7
Inc	RB	7	4 1	4	3 0	0	0	0	0
	VDRL	1	0 6	1	0 7	0	0	1	0 7
Neg	RB	107	63 1	90	67 3	121	72 0	97	68 4
	VDRL	122	71 6	98	73 2	112	66 6	99	69 8
Total		170		134		168		142	

untreated patients with early primary syphilis, with a serum titer of less than 4 units which have already been shown to yield equivocal results until the serum titer increases beyond 4 units, as the disease progresses[1]

About 75 per cent of all biologic false positive sera were from patients with experimentally induced malaria§ The results obtained with the series of known biologic false positive human sera are given in Table II Six categories of serologic reactions were established for purposes of evaluation (column 1):

B T denotes "biologic false positive type" and refers to complete inhibition of the serologic activity of euglobulin fractions in the presence of the inhibitor

§ These sera were obtained through the efforts of Mr John F Kent.

B T ? denotes "suggestive of biologic false positive type" and refers to low-titer euglobulin fractions (1 unit and less) ||

Inc denotes "inconclusive results" and includes euglobulin fractions which gave equivocal degrees of inhibition

Neg denotes "negative euglobulin fractions," regardless of the serologic titers of the whole serum

The two types of cardiolipin antigen emulsions are considered separately, i e RB (Rein Bossak) and VDRL cardiolipin antigens

The results were separately analyzed for each of the four participating laboratories (denoted by Roman numbers) Inspection of Table II reveals some variations among the results obtained in each of the four laboratories with regard to the per cent distribution among the six categories of serologic types These variations are due in part to variations in serologic activities of the euglobulin fractions, thereby shifting the results from S T *to* S T ?, *or from* B T *to* B T ? *or even to neg However,* variations were in part also due to definitely contradictory results, one laboratory reporting a B T reaction for a serum on which another laboratory reported an S T. or S T ? reaction Variations in titer were the more frequent of these two causes for discrepancies

A detailed analysis of the results will be made upon completion of this study It may be stated at this time that on the average about 3 per cent of all biologic false positive sera gave reactions which were either of the syphilitic type or suggestive of the syphilitic type, and about 1 to 2 per cent of the sera of this group gave inconclusive results Thus, the euglobulin-inhibition test has failed in about 5 per cent of all sera of biologic false positive origin About 70 per cent of these sera gave negative results, i e the euglobulin fraction being serologically inactive

An analogous analysis of the known syphilitic human sera is given in Table III The same considerations apply to this group of sera with respect to deviations among the four laboratories as were made above for the biologic false positive group of sera On the average, 5 to 6 per cent of all sera tested gave

|| The differentiation between B T and B T ? reactions and between S T and S T ? reactions has been tentatively introduced in order to evaluate in this study the influence of titer and of degrees of inhibition [illegible] his finer differentiation [illegible] T and S T reactions [illegible] of the minimal degree [illegible] T and S T reactions.

TABLE III

Known Syphilitic Human Sera

Serologic Type	Antigen	Laboratory I No	I %	II No	II %	III No	III %	IV No	IV %
S T	RB	66	77 8	50	83 3	46	67 7	53	68 8
	VDRL	66	77 8	44	73 3	28	41 2	34	44 2
S T ?	RB	8	9 3	5	8 3	4	4 8	5	6 5
	VDRL	8	9 3	6	10 0	10	14 6	11	14 3
B T	RB	2	2 4	0	0	4	5 9	2	2 6
	VDRL	1	1 2	0	0	3	4 4	2	2 6
B T ?	RB	2	2 4	1	1 7	4	5 9	2	2 6
	VDRL	0	0	2	3 4	4	5 9	3	3 9
Inc	RB	2	2 4	0	0	3	4 4	2	2 6
	VDRL	3	3 5	3	5 0	3	4 4	2	2 6
Neg	RB	5	5 9	4	6 7	7	10 3	13	16 9
	VDRL	7	8 1	5	8 3	20	29 5	25	32 4
Total		85		60		68		77	

serologic types of results which were definitely at variance with the clinical status of the patients from whom the sera were obtained, i e giving results of the biologic false positive type or suggestive of the biologic false positive type Since some of these sera were from patients who had received a full course of antisyphilitic therapy, it may be conjectured that the remaining serologic activity in these sera may be due to antibodies of the biologic false positive type, and that accordingly the biologic false positive type of reaction may be genuine However inviting such an explanation may be, it has not been considered in the evaluation of these results

Three to 4 per cent of the results obtained with the known syphilitic human sera were inconclusive, and 10 to 20 per cent were negative

The most rigorous interpretation of all the results which so far have been obtained in this survey places the specificity of the euglobulin inhibition test at a level of about 90 per cent

This is definitely the lower limit of resolving power because several of the causes which are responsible for equivocal results may be eliminated by a series of experimental improvements of the test which have been discussed elsewhere[1] These will be considered in more detail in the final report on the present cooperative study

At the present time, the euglobulin inhibition test is still being regarded as an experimental procedure and recommendations concerning its use for routine diagnostic purposes will have to be deferred until completion of this project

This project has been rendered possible by the unfailing cooperation and assistance of the other members of this research project Mr John F Kent, Dr Charles R Rein, and Mr Ad Harris It is a pleasure to record the author s thanks and indebtedness to these investigators A final report on this project will be made jointly by the entire group

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DISCUSSION OF PAPERS ON SEROLOGIC TESTS FOR SYPHILIS

Dr B S Kline (Mount Sinai Hospital of Cleveland) Work done with Eagle Hinton Kahn Kline and Mazzini antigens obtained from the originators and cardiolipin and lecithin solutions obtained from Dr Pangborn[4] in slide tests of cases of malaria showed false positive results with cardiolipin and lecithin in optimal proportions to be almost negligible (about 1%) whereas those with the other antigens averaged about 15%[*] Furthermore comparative slide tests with optimal cardiolipin lecithin antigen and with Kline antigen in a series of 27 000 tests showed the amazing result of only 7 false positive reactions in 24 000 non syphilitic sera with cardiolipin lecithin

REPORT OF THE CENTRAL STATISTICAL UNIT ON SCHEDULES INVOLVING AMORPHOUS PENICILLIN IN AQUEOUS SOLUTION

By

Margaret Merrell

Introduction

In the discussion following our report a year ago, this group raised a number of questions concerned with two issues: first, as to the effect on the failure rates of stiffening the criteria defining failures, particularly with regard to serological failures, and second as to the effect on the conclusions of pooling different kinds of cases, such as different stages of disease, both sexes, colors, etc., in determining the rates

I have therefore prepared the present report so that these issues may be examined To meet the first point I have determined the failure rates for clinical failures only That is, any case is considered a success until he is reported as a clinical failure This means that some persons previously classified as failures are for the purposes of this report classified as successes because they had a record of serological but not clinical failure, for others the time period when they are considered failures is later than in the previous classification since the clinical failure followed the serological, and for still others there is no change in the time or reason for assigned failure Although I am presenting figures only for clinical failures I shall indicate at some points how the comparisons would be altered if serological failures were included

With regard to the second issue, the cases have been separated into three groups, primary males secondary males, and secondary females It is recognized that it would be desirable to separate them also as to color and clinic The distributions of cases for these variables were determined, but it was impossible to estimate the failure rates specific for all these factors because the numbers were too small No rate was computed on less than 100 cases This means that it is necessary to have quite a substantial number treated in any given group in order that 100 should be still under observation and successful a year or more after treatment

The problem of the adequacy of follow up is always a difficult one It must always be remembered that there is no guarantee

that we are following a representative group of the cases treated It is entirely possible that those followed contain a higher proportion of clinical failure than would be found if everyone were observed One piece of evidence that makes us feel that this adverse selection is not too serious is that at the last point where rates were computed the distribution of cases followed as to color, sex, and initial diagnosis is just about the same as at the start In this report only schedules handled at clinics having a good follow up are presented

I am confining my report to schedules involving amorphous penicillin in aqueous solution Approximately 7,000 cases are included in the analysis All cases are brought up to date as of October 11, 1947, or to the period of latest observation Dr Rider will present the report from the Central Statistical Unit on penicillin in peanut oil beeswax and crystalline penicillin

RESULTS

Turning now to the actual rates, it will be well to look first at the clinical failure rates for all cases, irrespective of sex, race, and diagnosis, in order to get a view of the general level of the rates for different schedules

Figure 1 shows the cumulative clinical failure rates for all cases treated on 7 different schedule groups, ranging in total dose of penicillin from 0 3 million units to 4 8 million units At 15 months the rates range from 12% to 25% In general the rates improve with increased dose up to at least 1 2 million units The highest rate is for 3 million, the next highest for 6 million, and the next for a combined set of schedules involving 1 2 million The remaining four schedules involving 1 2, 2 4, and 4 8 million units at 3 hour intervals for 7 5 days, or in one case 15 days, are all together at 12 to 14% at 15 months

It is found that the total failure rates determined on first failure, whether serological or clinical, are approximately 1 5 times as large as the corresponding rates based on clinical failures only This relative change has the effect of separating the rates more widely, since the schedule having a 10% clinical failure rate has about a 15% total failure rate, whereas a schedule with a 20% clinical failure rate has about a 30% total failure rate Thus differences which are barely significant for clinical failures only may at times be significant for total failures and we can feel reasonably sure that any sizable difference found in the clinical rates will be substantiated and usually enhanced in the total rates

Fig 1

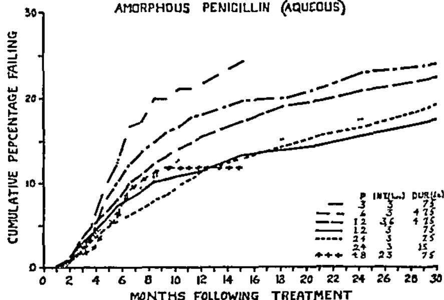


Table 1 shows the distribution of patients treated on these schedules as to sex, color and diagnosis There are some appre ciable differences as to kind of patients treated It may be seen, however, that schedules 01 and 06 (0 6 million units) are very similar in distribution to schedules 02, 13, and 14 (1 2 million), so the results may be compared The schedules with the larger dose show a maximum advantage in failure rate of only about 2% over those involving the smaller dose It will be noted also that schedules 07 (1 2 million) and 09 (2 4 million) have very similar distributions of patients Furthermore they are sched ules which are alike except as to size of dose They show virtu ally no difference in clinical failure rate

This evidence indicates that at about a year after treatment with amorphous penicillin in aqueous solution on any of these schedules at least 10% of the patients show clinical relapse or reinfection It indicates further that 1 2 to 2 4 million units give better results than lower doses It will be noted that all rates continue to rise over the entire period of observation

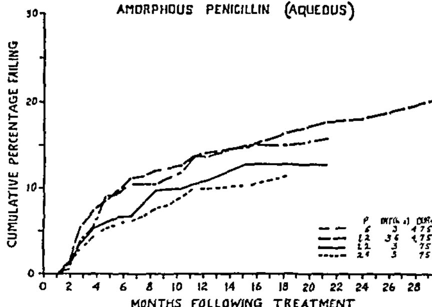
There are four of the schedules with doses from 0 6 million to 2 4 million units having large enough numbers of primary

TABLE 1

Distribution of Pat ents by Schedule Admission Diagnosis Color and Sex

	Schedule Number and Description						
	05	01 and 06	02 13 and 14	07	09	11	16 and 17
Amt P (mill un ts)	0 3	0 6	1 2	1 2	2 4	2 4	4 8
Interval (hrs)	3	3	3 6	3	3	3	2 3
Duration (da)	7 5	4 7 5	4. 7 5	7 5	7 5	15	7 5
Number of patients							
Treated	243	932	1,696	1 254	2 051	647	277
Followed 15 months	119	508	942	639	708	281	120
Percentage treated							
Total	100 0	100 0	100 0	100 0	100 0	100 0	100 0
Sero neg Primary							
White males	2 5	1 8	1 9	7 0	5 0	1 7	2 9
Colored males	5 8	5 8	6 2	8 4	5 7	2 5	2 2
Sero pos Primary							
White males	6 2	4 4	5 2	9 7	13 0	4 9	4 0
Colored males	9 9	12 9	11 9	16 0	13 2	6 6	5 8
Early Secondary							
White males	4 1	5 3	5 5	8 6	8 5	2 2	4 7
Colored males	12 8	17 4	15 7	11 9	13 5	4 2	13 7
Wh te females	0 4	8 2	7 4	7 2	7 1	10 2	6 1
Colored females	31 7	29 2	29 2	15 3	12 0	42 5	17 3
Other and unknown	26 6	15 0	17 0	15 9	22 0	25 2	43 3

Fig 2

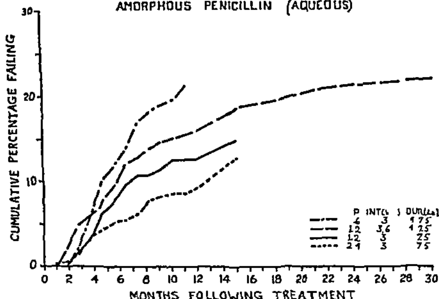


males to allow comparison Fig 2 shows the comparison of the rates None of them differs significantly at 15 months from ai other The rates for this group are only a little lower than t corresponding rates for all cases They range from 10 to 15 at 15 months, when the corresponding rates for all cases rang from 13 to nearly 20% at the same period

The numbers did not permit computation of rates speci for serological status and color, but referring back to Table for the distribution of the cases, it may be seen that about o third were sero negative, the proportion colored varied fro about 75% for the schedule with the lowest dose (0 6 millior to about 50% for the schedule with the highest dose (2 4 mi lion). If the colored have higher rates than the white, the failu rates would be brought still closer together if we adjusted th factor

The failure rates for secondary males for the same fou schedules are shown in Fig 3 This graph looks very differen from the preceding largely because of the increase in rates fo

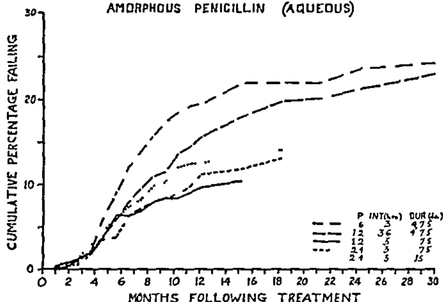
Fig 3



the least favorable schedule There is no increase at all in the 2 4 million unit schedule Various explanations may be thought of, which are worth further investigation One is that the small dose which might be adequate to deal with a new infection is inadequate to deal with a longer standing one, but that the larger dose is competent to handle either It is interesting too to see indication of some advantage of 2 4 million units over 1 2 million This advantage is shown still more definitely when serological failures are included

The clinical failure rates for secondary females (Fig 4) present an essentially similar picture to that for secondary males As before the lowest dose shows a definitely higher rate than the higher doses The schedules involving 1 2 and 2 4 million units are not sharply separated, the two schedules involving 1 2 being above and below those involving 2 4 The two of these schedules which appear less favorable have a considerably higher proportion of colored patients than the other two, which may account for the results

Fig 4
CUMULATIVE FAILURE RATES — SECONDARY FEMALES
CLINICAL FAILURES ONLY
AMORPHOUS PENICILLIN (AQUEOUS)



Discussion

Viewing this discussion as a whole we find that even when clinical failures alone are examined, at least 10% of the patients treated are classified as failures at the end of the first year on the most favorable of the amorphous aqueous penicillin sched ules considered here and for the most favorable diagnostic group These rates continue to climb gradually over the entire observa tion period Since we now have several schedules with substan tial numbers followed up to three years there is some possibility that an analysis of the results over this long period may throw some light on the difficult question of reinfection

The more rigid definition of failure demanding clinical signs does not alter in any material way the conclusions previously presented for all failures although it does minimize some of the differences found for total failures because of the smaller rates

On the other hand the consideration of the specific diagnostic groups sharpens up some differences which were only suggested when all cases were pooled In one group there was an indication that 2 4 million units had definite advantage over 1 2 million units, which had not been seen in previous comparisons Perhaps

the greatest value to the study of specific groups is that it has suggested a number of other possible leads which we hope to pursue relative to some of the questions involved in the continued rise in failure rates

THE THERAPEUTIC EFFICACY OF AQUEOUS PENICILLIN IN EARLY SYPHILIS

By

John F Mahoney, Robert D Wright, and *John A Trautman**

In the present report some of the essential features of the various schedules for penicillin treatment of early syphilis, which have been employed in the Venereal Disease Research Laboratory during the past five years, will be reviewed Also, a preliminary statement about a penicillin routine in which the duration of therapy has been reduced to three days will be given

Only patients with early syphilis have been included in any of the schedules In all instances the diagnosis was established beyond any reasonable doubt All patients classed as seronegative are negative to all recognized tests for syphilis as well as free from all evidence of clinical disease A regrettable, but apparently entirely unavoidable, loss of patient material from post-treatment observation is reflected in all of the treatment groups This probably is due to the nomadic tendency among seafaring men who comprise the larger part of the patient group Commercially available penicillins of various manufacturers were employed

The initial treatment schedule as listed in Table 1 was 1 2 million units of the antibiotic given in 20,000 unit doses at 3 hour intervals for 60 injections The total treatment period was 7½ days This original schedule was a fortunate choice since it supplied the basis for the study of more intensive schedules At no time were schedules of lesser intensity employed

It may be of interest to recall that the original group of four patients the treatment of whom formed the basis of the first report on the penicillin therapy of syphilis[1], were treated with 1 2 million units It may be of general interest also that these four patients are still under observation and that, as they pass into the sixth year, all are seronegative and free from clinical evidence of the disease

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A total of 92 patients were treated by the original schedule Without attempting to break the data down into precise categories, it may be stated that a total of 61 of the group attained seronegativity It was considered advisable to re-treat 17 patients This included individuals re treated for any reason serorelapse, serofailure, clinical relapse, or reinfection Of the re treated group nine have been observed to reach the negative phase, and an additional seven were progressing in a satisfactory manner when last observed The results of this treatment schedule, which was in the nature of a preliminary trial, were considered to be highly informative

As soon as the re treatment rate in the initial schedule had reached a reasonable limit, the study of the second schedule was undertaken This schedule employed 40 000 units of penicillin injected at 3 hour intervals for a total of 60 injections The total penicillin was 2 4 million units and the period of treatment was 7½ days There were 98 patients in this group, of whom 74 attained seronegativity Ten were re treated for some one of several reasons, the remaining patients were lost from observation while in the indefinite serologic status Of the ten re treated patients five attained seronegativity and four were progressing in a satisfactory way when last observed

In the third treatment schedule a basic change was made The interval between injections was reduced to two hours and the number of injections increased to keep the duration of therapy at about 7½ days

The results of the administration of penicillin at 2 hour intervals were studied in three groups of patients, approximately 1 100 in all The first two groups were treated with total penicillin dosages of 1 5 million and 1 7 million units, respectively

The group considered to be of greatest importance was 728 patients who received 40 000 units of penicillin on a 2 hour basis for a total of 85 injections The findings in this group have been published in detail[2] Of the group it was considered advisable to re treat a total of 25 individuals Although it was felt that a large proportion of these represented new infections, all have been grouped in the re treatment category Five of the re treated patients had attained seronegativity and 13 were progressing satisfactorily when last observed On the whole, this schedule produced results which prompted the conclusion that a desirable improvement of the therapy would be to reduce the time period during which the patient is hospitalized The last schedule, now under study, has this objective in view

The final schedule involves the use of 7 2 million units of the antibiotic administered in injections of 200,000 units at 2 hour intervals for a total of 36 injections This reduced the period of hospitalization to three days, instead of the 7½ days required by the previous schedule The details of this study are indicated in the following table

Of the total of 491 patients, a considerable loss of material, caused by failure of posttreatment observation, is indicated Many of those apparently lost from the study will be encountered at a later date

In the initial observation period, 126 of the patients attained seronegativity and seven were re treated for one of the usual reasons The remainder were making satisfactory progress in accordance with the criteria which had been developed in the previous studies That five of the re treated cases were the result of reinfection is the measured opinion of the clinicians who were in a position to assay all factors concerned with each individual instance

In the second observation period interest centers in the fact that the ratio of seronegative patients increases in relation to the total number observed This feature has been noted in other study groups The longer the posttreatment period of observation, the higher becomes the proportion of individuals who attain seronegativity In this period an additional five patients were re treated All were considered to be reinfected

In the observation period extending up to the beginning of the second year, all but 19 of those observed had attained seronegativity and an additional four, in whom, again, the evidence pointed to reinfection, had been re-treated

Although a detailed comparison of the most recent schedule with the most successful of the 7½ day schedules is not possible, the general characteristics of the patterns are similar If the continued observation of the patients fails to reveal evidence of relapse or of treatment failure in a proportion greater than has been encountered in previous groups, the inference will be warranted that the curtailed treatment period may be safely substituted for the longer period There will remain to be worked out a more precise penicillin dosage The present level of 7 2 million units may be, and probably is, excessive

From the public health aspect, the ideal type of antisyphilitic therapy is one which may be employed in ambulatory patients, with the clinic or rapid treatment center serving as the focus for diagnosis and treatment According to the present state of

TABLE I

Serologic Status of Patients at Last Observation Who Received Penicillin Therapy for Early Syphilis

Schedule	Total Treated	Not Observed	Indefinite	Improved	Negative	RE-TREATED Not Observed	RE-TREATED Indefinite	RE-TREATED Improved	RE-TREATED Negative	Number Re-treated
20M x 60	92	12	—	2	61	1	—	7	9	17
40M x 60	98	5	—	9	74	1	—	4	5	10
20M x 75	212	62	1	18	108	6	2	10	5	23
20M x 85	459	141	3	34	237	13	2	19	10	34
40M x 85	728	180	1	105	417	7	—	13	5	25
200M x 36	491	111	6	150	208	8	1	4	3	16

knowledge only a repository type of preparation can function in this way It is hoped that more adaptable and more consistently reliable preparations than those developed up to the present will make their appearance Such an eventuality would greatly enhance the control program In the meantime, if hospitalization is to be required, the 3-day routine represents an appreciable saving for a health department

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REPORTS OF THE CENTRAL STATISTICAL UNIT

II AMORPHOUS PENICILLIN VERSUS CRYSTALLINE PENICILLIN G, III AQUEOUS PENICILLIN VERSUS PENICILLIN-OIL-BEESWAX

By
*Rowland V Rider**

Most of the comparisons that will be made in this paper between amorphous penicillin and crystalline penicillin G, and between the aqueous and the peanut oil beeswax vehicles will be based on cumulative failure rates The definitions of failure used will be mentioned when specific comparisons are being made Other assumptions and definitions have been detailed in earlier reports of the Central Statistical Unit

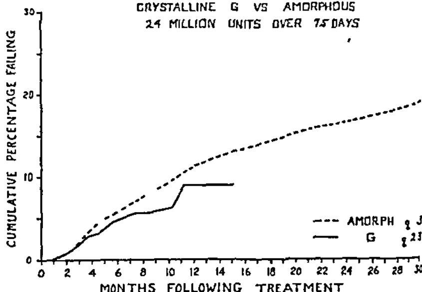
Sufficient data for statistical analysis have been reported on four schedules using crystalline penicillin G Two schedules called for 2 4 million units to be given over a period of about 7 5 days One of these had a 2 hour interval between injections, the other a 3 hour interval For each, about 650 cases were treated and reported

The other two schedules are similar as to duration of treatment and interval between injection, but call for twice the amount of penicillin, i e, 4 8 million units Slightly over 800 cases were treated and reported for each of these schedules

The 2 4 million unit schedules will be examined first There were four schedules in the study which used 2 4 million units of amorphous penicillin One of these, using a peanut oil-beeswax vehicle, is unsuitable for comparison with the crystalline G

* Department of Biostatistics The Johns Hopkins University School of Hygiene and Public Health

schedules both because of insufficient follow up and incompara intervals of injection The other three 2 4 amorphous schedu involved 3 hour intervals between injections and total durati of treatment of 4, 7 5, and 15 days They exhibited similar f ure rates To make the comparison between crystalline G amorphous as specific as possible, then the 2 4 amorphous sch ule using 7 5 days duration will be used



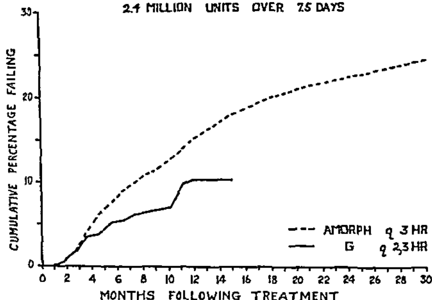
If clinical failures only are included no very marked diffe ences between the amorphous and the crystalline G appear Th broken line represents the results obtained with amorpho penicillin, the continuous line with crystalline G For this co parison the two crystalline G schedules involving 2 4 mill units have been combined The combination seems justifie because the results for these schedules were not significan different and the cases treated in the two schedules have simil distributions of race, sex, treatment diagnosis, and clinic

If, in addition to cases observed to fail clinically, we includ among the failures serorelapses and seroresistants for wh clinical failure was not observed we find a much more mark difference between amorphous and crystalline G

Again the broken line is for the amorphous The size of this difference is so great that some explanation other than chance must be sought for it

The clinics contributing to the amorphous group differ somewhat from those treating with crystalline G, but correction for this would make the observed difference even greater

This is true also in the case of race differences, since in the amorphous group the ratio of white to colored cases is two to one, while in the crystalline G group the ratio of white to colored cases is one to three

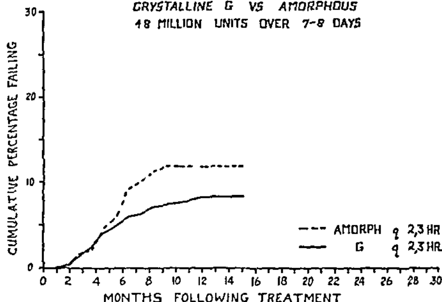


The diagnosis differences are not great and are of a balancing sort and so can not be credited with an influential contribution to the observed differences in failure rate

In the amorphous group there were 2 males for every 3 females, whereas in the crystalline G group this ratio is reversed This is the only apparent difference, a correction for which might reduce the discrepancy between the G and amorphous failure rates However, an examination of the sex specific rates for the amorphous schedule shows no difference so that identical proportions of each sex in the G and amorphous groups would not change the comparisor

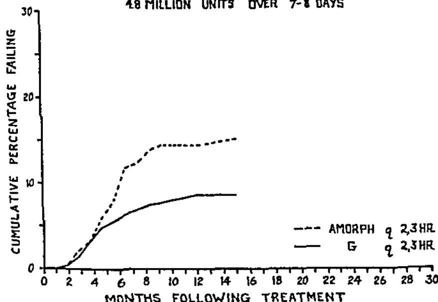
This comparison, then, offers strong evidence that results obtained by crystalline G are superior to those obtained by amorphous penicillin up to the fourteenth month following treatment

We find the same sort of thing when the crystalline G schedules using 4 8 million units are compared with amorphous schedules using identical total dosage, duration of treatment, and intervals between injections



The rates in this comparison are based on clinical failures only and show roughly the same size difference as was shown by the 2 4 schedules The broken line represents a combination of two amorphous schedules, the continuous line the two crystalline G schedules The combinations are justified on the same grounds that were mentioned concerning the 2 4 million unit schedules

Looking at the same groups of cases, but this time with the definition of failure including serorelapse and resistance we find, as we did with the 2 4 schedules, a difference that is unlikely to be due to chance The sex, race, treatment diagnosis and clinic differences between the amorphous and crystalline G are too slight or in the wrong direction to account for this difference



The conclusion may reasonably be drawn that up to the fourteenth month following treatment penicillin crystalline G, when given in quantities of 2 4 or 4 8 million units over a 7 5 day period at the rate of an injection every 2 or 3 hours, gives results superior to those obtained by amorphous penicillin of comparable dosage

It might be pointed out that the evidence at hand is insufficient to indicate any difference between 2 4 and 4 8 million units of G, or between the 2 and 3 hour intervals between injection.

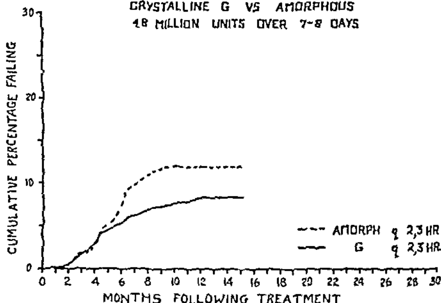
There were no consistent differences between the G and the amorphous regarding reactions to the treatment as these were reported

Turning now to a comparison of vehicles, analysis of the reports received on the use of amorphous penicillin does not justify the conclusion that there is any difference between the cumulative failure rates obtained by the use of the peanut oil-beeswax vehicle and the rates observed among cases treated with penicillin in aqueous solutions

Two schedules using peanut oil-beeswax used a total dosage of 4 8 million units of amorphous penicillin over an 8 day period

This comparison, then, offers strong evidence that results obtained by crystalline G are superior to those obtained by amorphous penicillin up to the fourteenth month following treatment.

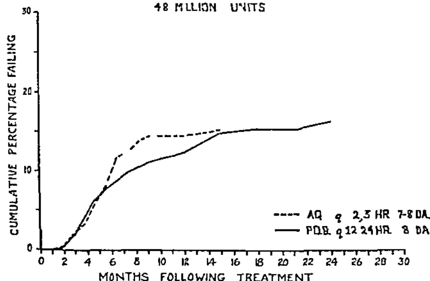
We find the same sort of thing when the crystalline G schedules using 4 8 million units are compared with amorphous schedules using *identical total dosage, duration of treatment,* and intervals between injections



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Looking at the same groups of cases, but this time with the definition of failure including serorelapse and resistance we find, as we did with the 2 4 schedules, a difference that is unlikely to be due to chance The sex, race, treatment diagnosis and clinic differences between the amorphous and crystalline G are too slight or in the wrong direction to account for this difference

By combining the two aqueous schedules this comparison may be carried to the fourteenth month following treatment The continuous line represents the two P O B schedules Only clinical failures were used in this comparison



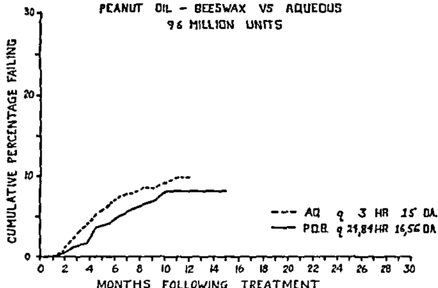
This shows the results if serologic failures not accompanied by clinical failures are included Thus by the fourteenth month no difference between the P O B and the aqueous is detectable on the basis of cumulative failure rates

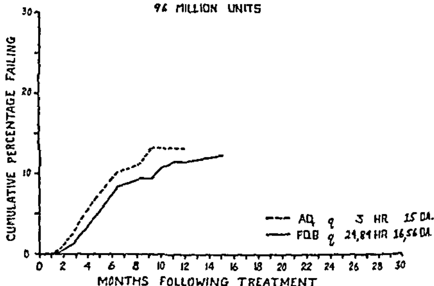
Examining the P O B schedules using 9 6 million units by a comparison with a single schedule using the same total dosage in aqueous solution we arrive at the same conclusion, whether clinical failures only are included, or all failures

The conclusion of previous reports remains unchanged, namely, P O B does at least as well as aqueous penicillin

In the over all reaction rates there is no difference between the particular P O B and aqueous schedules that we have been discussing There is some indication that abscesses at the point of injection are more common when P O B is used However, only six cases were reported out of 1597 cases with reaction information No such cases were reported in the smaller aqueous group here considered Also, among the P O B cases two or three cases of pain and swelling in the region of the injection

were mentioned against none from the aqueous group. The incidence of Herxheimer reactions was about the same in each group.





TREATMENT FAILURES VERSUS REINFECTIONS FOLLOWING PENICILLIN THERAPY IN EARLY SYPHILIS

By

R C Arnold and *F P Nicholson**

Our conception of syphilis as an infectious disease can be clearly set forth by analogy with another infectious disease—malaria Each has a specific therapy, penicillin and quinine, respectively, which cures if given in adequate amounts Inadequate treatment results in relapses or in similar states of latency or chronicity We should expect, then, that the host of early syphilis who has been cured can be reinfected as can the successfully treated malarial patient

Syphilis differs from malaria, perhaps, in being more capable of arousing the host s immune response If treatment is delayed until these processes are well established, then reinfection after adequate therapy may not take place until the resulting immunity has disappeared In some instances, the existing local tissue immunity may adversely affect the spirochetal invasion so that a symptomless reinfection will occur

Since the advent of rapidly completed antisyphilitic therapies, especially in the half decade of penicillin usage, reinfections have been noted with increasing frequency Serologic or clinical relapses were observed too frequently in the early experimental schedules but, as the adequate time dose relationships were determined, the relapse rate steadily declined Relapses and reinfections assume major roles in the scientific studies designed to evaluate the values of antisyphilitic therapies A relapse is a true treatment failure A reinfection is an immune or prophylactic failure and should not adversely affect the efficacy of therapy The differentiation of relapse from reinfection has been considered to be a difficult and controversial subject but the solution of the problem should be approached by an unbiased evaluation of the experimental, clinical and laboratory data related to all phases of the disease

The primary premise of reinfection is The host has experienced a previous infection from which it has completely recovered In previous publications it was demonstrated that reinfection could be produced in animals with acute or latent syphilis

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within ten days after the completion of adequate penicillin therapy The manifestations of the disease resulting from the second spirochetal invasion varied with the duration of the first infection and the subsequent development of local and systemic immune forces by the host All animals adequately treated for early syphilis could be reinfected, yet only 27 per cent developed a new lesion at the site of the second induced invasion It has since been demonstrated that the chancre of the reinfection may occur at the site of the original chancre Symptomless invasion was also noted in 53 per cent of the animals treated for latent syphilis, the remainder were immune to the second invasion of the *Spirochaeta pallida* Since symptomatic and asymptomatic reinfection may occur and since the symptomatic reinfection may resemble a serologic relapse, the differentiation is difficult unless the serologic pattern yields significant data

If the clinical and positive serologic manifestations of the syphilitic host disappear after therapy and if the seronegative host experiences a second darkfield positive chancre, then it is generally conceded that a reinfection occurred Examples of this type of invasion and subsequent reinfection have been observed as early as one or two days and as late as two years after the completion of penicillin therapy Since symptomatic reinfections occur in the human host, it is reasonable to assume that asymptomatic reinfections also occur

The serologic manifestations of relapses and reinfections have been studied to see if the quantitative tests portray characteristic patterns for each condition It was observed in the patients that had developed serologic and clinical relapse or only serologic relapse that the relapsing titer levels off at a point below the original titer On the other hand if the host experiences a second syphilitic infection or a nonspecific stimulus, the serologic titer rises rapidly to a higher peak than was observed with the original infection Obviously if retreatment is started immediately after the development of the second darkfield positive chancre, the titer may not have ample time to reach the higher levels

Chart 1 illustrates a serologic relapse, plateau formation, and successful therapeutic response A patient with early secondary syphilis had a pretreatment Kahn titer of 64 dils (dil — the highest dilution in which a positive result is obtained) The inadequate therapy given the patient reversed the titer to the negative phase in 12 weeks, but subsequently a definite serorelapse (treatment failure) developed In the 18th week the

titer relapsed to 16 dils, thereby forming a plateau which was maintained until the 25th week At that time adequate penicillin therapy was given which was followed by a reversal to the seronegative state which has persisted without interruption

CHART 1

SEROLOGIC RESPONSE IN PENICILLIN TREATMENT OF SYPHILIS

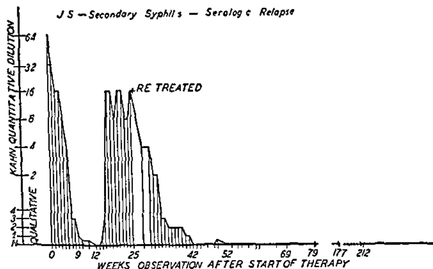
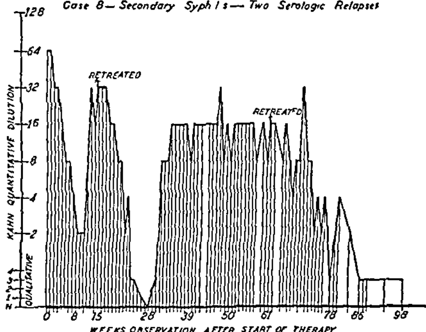


Chart 2 illustrates two serologic failures before successful treatment A patient with secondary syphilis had a pretreatment Kahn titer of 64 dils After inadequate therapy the titer declined gradually in 8 weeks to 2 dils There was a subsequent relapse to the 32 dil level which was observed for 3 weeks A second inadequate course of therapy caused the titer to decrease to the negative phase which was followed by the second serologic relapse to the 16 dil level This second plateau was observed for a total of 27 weeks A third, and adequate, course of penicillin was given, after which the titer gradually approached and finally reached the negative phase on the 193rd week (not shown on slide) It is interesting to note that the levels of the titers were different for each serologic relapse, but at no time did the titer rise above or equal the original pretreatment level If this patient had been given an adequate dosage of penicillin after the first relapse the favorable serologic response which was noted would probably have continued to the negative phase Also if adequate therapy had been given as soon as the second relapse was evident, the response might have taken place more rapidly It is believed



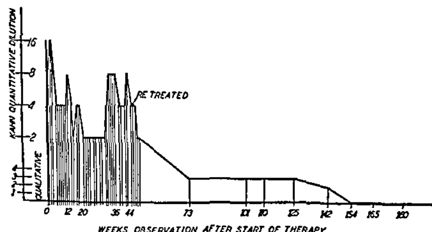
that certain host factors influencing penicillin utilization by the body, instead of strain resistance, were responsible for the treatment failures Another patient infected with the same strain of *Spirochaeta pallida* received the same original inadequate dosage and subsequently developed a serologic relapse The second course of penicillin therapy was the same for both individuals, yet only one responded in a satisfactory manner

Chart 3 demonstrates serologic failure in very early latent syphilis The patient had a pretreatment Kahn titer of 16 dils. Following inadequate therapy, the titer declined to an 8- or 4-dil level which persisted for 44 weeks A second penicillin regime was given on the 44th week, after which the titer gradually decreased and finally reached the negative phase on the 154th week With quantitative tests, in treated syphilis a one tube fluctuation above or below the plateau may be expected

Chart 4 demonstrates primary syphilis and subsequent reinfection (primary type) The patient was treated with penicillin for early primary syphilis The highest Kahn reaction was a 3 plus qualitative result which declined to the negative phase in 4 weeks The serologic pattern remained negative until the

CHART 3

LCA – EARLY LATENT SYPHILIS – SEROLOGIC FAILURE



9th month when the patient experienced a second infection which produced a dramatic and significant rise in serologic reactivity During the period of treatment the peak of 128 dils was maintained Serologic response to penicillin therapy for the reinfection was prolonged, but eventually on the 30th month the negative state was reached and was maintained to the 52nd month

CHART 4

SEROLOGIC RESPONSE IN PENICILLIN THERAPY

S.L Primary Syphilis – Reinfection

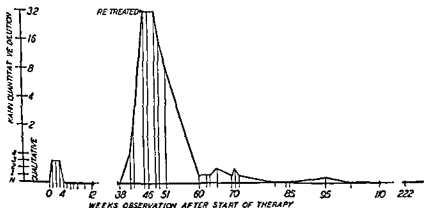
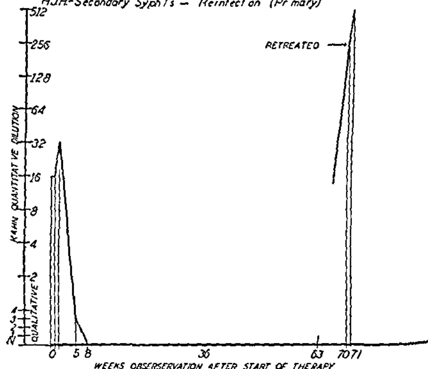


Chart 5 illustrates the serologic pattern in secondary syphilis and reinfection (primary type) The patient had secondary syphilis with a Kahn titer of 32 dils Following therapy, the seronegative phase was reached in 8 weeks and remained so until the 70th week At this time the host experienced a primary reinfection which produced an explosive serologic reaction which attained 256 dils on the 21st day After treatment it was possible to follow the subsequent decline for only one week

Chart 5

SEROLOGIC RESPONSE IN PENICILLIN TREATMENT OF SYPHILIS

HJH-Secondary Syphilis — Reinfection (Primary)



It has been noted that nonspecific stimuli e g, small pox vaccination, may produce serologic patterns that resemble the ones of syphilitic reinfections The posttreatment observations and the clinical studies yield clarifying data for the nonspecific serologic manifestations

PENICILLIN IN EARLY SYPHILIS: AN ANALYSIS OF THE DISCREPANCIES BETWEEN THE RESULTS OF ARNOLD ET AL AND THOSE OF THE CENTRAL STATISTICAL UNIT

By
Frank W. Reynolds†

In November 1947, Arnold and his co-workers[1] at the Venereal Disease Research Laboratory in Staten Island published a report of their experience with penicillin in early syphilis Their results, so modestly presented, have aroused considerable interest because they are significantly superior to any previously reported either by the Central Statistical Unit[2] for the clinics cooperating in the nation-wide penicillin study, or by others

Having been given access* to the data from the Central Statistical Unit, as well as from the U S Marine Hospital at Staten Island, it has been possible to make certain observations and comparisons that tend to clarify the apparent discrepancy between the results of the two groups

(1) *Definition of Terms* It is first to be noted that the data of Arnold et al are based upon cumulative *retreatment* rates, whereas those of the Central Statistical Unit are in terms of cumulative *failure* rates The two are not synonymous The Staten Island group reserves retreatment for those patients who are observed to have either overt clinical relapse (or reinfection) or indubitable serologic relapse, the latter based upon the results of a battery of carefully controlled serologic tests, thus minimizing technical variations in any one procedure The Statistical Unit has considered as failures not only these two groups, but also patients with "non conforming" serologic relapses (based on the results of one test rather than on a battery of tests), as well as a group who "fail" because of being seroresistant

In order to determine the degree to which this difference in definition of terms (and serologic procedures) influences the outcome, appropriate Statistical Unit schedules** were rean-

* Through the courtesy and cooperation of Dr Margaret Merrell Dr Rowland Rider, and Miss Gwendolyn Futcher of the Central Statistical Unit, and of Dr John F Mahoney, Director Venereal Disease Research Laboratory, U S Marine Hospital, Staten Island

** These schedules were selected as being most like that of Arnold et al The details are as shown below

† Division of Research Grants and Fellowships, National Institute of Health

TABLE I

PENICILLIN IN EARLY SYPHILIS COMPARATIVE RESULTS OF ARNOLD ET AL AND THE CENTRAL STATISTICAL UNIT

	Arnold et al	Central Statistical Unit			
	3 4 q 2 hr 7½ days	(A) 4 8 q 2 hr 8 days	(B) 4 8 q 3 hr 8 days	(C) 9 6 q 3 hr 15 days	(A) (B) & (C) Combined
No of Clinics	1	3	9	9	16
Pts Treated	728	143	134	290	567
Days After Treatment					
1 – 30	0 00	0 00	0 00	0 00	0 00
31 – 60	0 00	1 14	0 12	1 02	0 59
61 – 91	0 41	3 88	1 23	3 13	2 25
92 – 121	1 06	7 18	2 76	6 07	4 62
122 – 151	1 52	10 84	4 03	8 60	7 34
152 – 182	2 00		8 82	10 81	8 98
183 – 212	2 00			12 11	12 67
213 – 242	2 29			12 48	13 19
243 – 273	2 29			14 07	14 28
274 – 303	2 66			14 53	15 42
304 – 333	3 08			15 19	15 42
334 – 365	3 55			15 27	16 08
366 – 395	3 55				16 43
396 – 425	4 75				18 08

alysed on the basis of the Staten Island criteria It was possible, among a group of 566 patients, to exclude 19 from the group of 75 'failures" Representative of those reclassified are the following (Table II)

A recomputation of the Central Statistical Unit data based on Staten Island criteria (Table III) indicates that it is possible to reduce substantially the cumulative failure rate, but that there still is a significantly higher percentage of failures than reported by Arnold and his co-workers

*(2) Differences in Patient Population There are obvious dif-*ferences in the patient populations treated at Staten Island and those treated by the cooperating clinics In Table IV are shown the race, sex and stage of disease distributions in the two groups Especially noteworthy is the fact that the Staten Island group of patients consists largely of white males with primary syphilis, whereas the largest single group of CSU patients is made up of Negro females with secondary syphilis

TABLE II

REPRESENTATIVE CASES ORIGINALLY CLASSIFIED AS FAILURES BY C S U RECLASSIFIED AS NON FAILURES

CSU No	Sts	Months After Treatment 0	1	2	3	4	5	6	7	8	9	10	11	12
110054	Kahn Un ts	48	2	1	0	0	0	——	8	16	0	0		
349825	Kahn Un ts	0	0	0	0	3	32	0	1	0	0	0	0	0
090142	Kahn Un ts	128	32	32	2	3	4	4						
340128	Kahn Un ts	16	32	0	0	64	——	——	——	——	——	——	0	
010635	Kahn Un ts	128	0	0	0	0	0	3	1					
029078	Kahn Un ts	0	0	40	4	1	1	3	0	0	0	0		

TABLE III

RECOMPUTATION OF CENTRAL STATISTICAL UNIT DATA ON BASIS OF STATEN ISLAND CRITERIA

Days After Treatment	Total	No Observed This Period or Later	CSU Criteria		Staten Island Criteria	
			Failures	Cumul % Failures	Failures	Cumul % Failures
0	35					
0 – 28	19	531	0	0	0	0
29 – 56	32	512	3	59	3	59
57 – 84	30	480	8	2 25	8	2 25
85 – 112	27	450	11	4 62	8	3 99
113 – 140	24	423	12	7 34	10	6 26
141 – 168	25	399	7	8 98	5	7 43
169 – 196	30	374	15	12 67	11	10 15
197 – 224	18	344	2	13 19	0	10 15
225 – 252	21	326	4	14 28	3	10 98
253 – 280	24	305	4	15 42	4	12 15
281 – 308	24	281	0	15 42	0	12 15
309 – 336	16	257	2	16 08	1	12 49
337 – 364	37	241	1	16 43	1	12 85
365 – 456	123	204	4	18 08	0	12 85
457 – 548	37	81	1		1	
549 – 639	17	44	0		0	
640 – 730	15	27	1		1	
731 – 912	12	12	0		0	
Totals	566		75		56	

It is difficult to demonstrate in the CSU material any statistically valid difference between the races, the sexes or between primary and secondary syphilis (Table V). There are, nevertheless, trends favoring whites, males, and these in the primary stage of the disease When a comparison is made of white males with primary syphilis versus Negro females with secondary syphilis (Fig 1), there is a difference that could occur by chance alone but once in 267 times $(x/\delta = 2\ 90)$.

There can be, therefore, little question but that the results of Arnold and his co-workers are influenced favorably by the type of patients they have treated

(3) *Differences in Follow-up* (a) Success —It has been presumed that patients who are lost from post-treatment observation are cured or fail of cure at the same rate as do those who remain under observation There is some indirect evidence that this is not the case CSU clinics whose follow-up is good have had quite uniformly higher failure rates than have those whose follow-up record is less satisfactory

It is perhaps of some significance that the follow-up of pa-

TABLE IV

SEX, RACE AND STAGE OF DISEASE DISTRIBUTIONS OF PATIENTS IN C S U. AND STATEN ISLAND SERIES

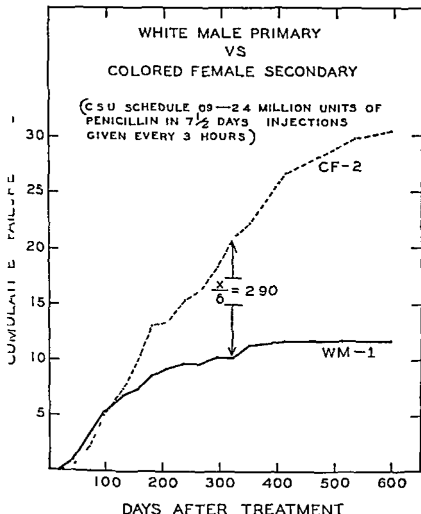
Arnold et al (728 Patients)								Central Statistical Unit (566 Patients)							
Male				Female				Male				Female			
White		Negro		White		Negro		White		Negro		White		Negro	
1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°	1°	2°
53 7%	22 8%	11 4%	8 6%	1 2%	1 5%	3%	5%	10 8%	7 4%	14 7%	14 3%	4 3%	10 0%	7 9%	30 6%
76 5%		20 0%		2 7%		0 8%		18 2%		29 0%		14 3%		38 5%	
Male 96 5%				Female 3 5%				Male 47 2%				Female 52 8%			
White 79 2%				Negro 20 8%				White 32 5%				Negro 67 5%			
Primary 66 7%				Secondary 33 3%				Primary 37 7%				Secondary 62 3%			

TABLE V

SEX, RACE AND STAGE OF DISEASF AS FACTORS INFLUENCING FAILURE RATES AFTER PENICILLIN THERAPY (CSU DATA)

Days After Rx	CUMULATIVE PERCENTAGE FAILING					
	Primary	Secondary	White	Negro	Male	Female
0	0	0	0	0	0	0
0 - 28	0	0	0	0	0	0
29 - 56	58	66	65	56	83	37
57 - 84	3 68	1 70	3 35	1 76	2 15	2 35
85 - 112	6 36	3 15	4 82	3 63	4 00	4 00
113 - 140	8 48	5 79	5 59	6 21	5 94	6 15
141 - 168	8 48	7 74	6 40	7 54	6 45	7 92
169 - 196	9 27	11 82	8 11 *	10 68 *	8 54	11 23
197 - 224	9 27 *	11 82 *	8 11	10 68	8 54	11 23
225 - 252	10 20	12 72	8 11	11 81	9 15	12 24
253 - 280	10 20	14 59	8 11	13 40	9 81	13 82
281 - 308	10 20	14 59	8 11	13 40	9 81	13 82
309 - 336	10 20	15 12	8 11	13 88	9 81	14 41
337 - 364	11 58	15 12	8 11	14 39	10 68 *	14 41 *
365 - 456	11 58	15 12	8 11	14 39	10 68	14 41
	* x/δ = 0 78		* x/δ = 0 83		* x/δ = 1 08	

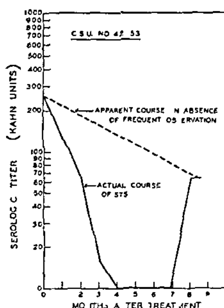
Fig 1

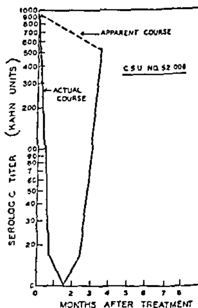


tients treated at the Staten Island Marine Hospital necessarily is relatively poor, for the reason that most of the patients are merchant seamen and difficult to keep under observation Approximately 30 per cent of the patients are lost from observation as soon as they leave the hospital, and at the end of one year, only slightly more than one-third are still available for follow-up

(b) *Frequency of post-treatment observations* —There is in the material of the CSU not a few patients whose serologic course shows an early decrease in titer followed by an increase (serologic relapse), the latter frequently not as high as the pretreatment level It is possible that some of the Staten Island patients

with "unchanged or improved serologic status" have followed such a course and might have been classed as serologic relapses had more frequent observations been possible





(4) *Other Possible Factors* There are other possible factors It has been suggested that merchant seamen may be less likely to be reinfected especially from their original contacts , It also has been suggested that additional anti syphilitic therapy in some instances may be given to merchant seamen, since their travels bring them in contact with physicians who may not be cognizant of newer methods of syphilotherapy What part, if any these factors play is pure conjecture

Discussion It will be noted that all of the factors tend to favor minimal failure rates in the Staten Island series, and to emphasize the incidence of failures in the cooperating clinics

Arnold and his co-workers imply a special virtue in the administration of penicillin every two hours Schoch[3] also has found the two hour interval to be more satisfactory than schedules involving injections every three hours On the other hand, Thomas[4] has observed no significant difference between two and three hour injection intervals Moreover, the experimental work of Eagle Magnuson and Fleischman and the clinical data of the Central Statistical Unit suggest no advantage in shortening the time interval between injections

Whether there is any inherent virtue in the two hour schedule in be neither affirmed nor categorically denied from the evience here adduced It does seem, however, that the apparent iscrepancy between the results of the Staten Island group and hose of the Central Statistical Unit can be resolved without takig into account this variable

eferences

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(2) Merrell, M Results of the Nation wide Study of Early Syphilis aper read at Syphilis Study Section Symposium, Washington, D C, April *l*, 1947

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THE JARISCH HERXHEIMER REACTION IN EARLY SYPHILIS TREATED WITH CRYSTALLINE PENICILLIN G*

By

Thomas W Farmer, M D **

The transient intensification of the symptoms and signs of arly syphilis shortly after the institution of therapy (i e the Jarisch Herxheimer reaction) has been repeatedly observed after mercury, bismuth and arsenical drugs In 1943 Mahoney, Arnold and Harris first described the same febrile and cutaneous reactions in patients with early syphilis within eight hours after the initial injection of commercial penicillin

The usual symptoms and signs of the Herxheimer reaction in early syphilis include any one or combination of the following

1 Systemic or febrile phase of the reaction chilliness, ele vation of temperature malaise, headache, and nausea

2 Focal or cutaneous phase of the reaction edema or pain in the primary ulcer, enlargement of or pain in the regional lymph nodes, intensification of a secondary eruption or of other

* From the Johns Hopkins University and the United States Public Health Service Venereal Disease Research and Post-Graduate Training Center

* This work was done under a grant from the Research Grants and Fellowships Division National Institute of Health United States Public Health Service

** National Institute of Health Senior Fellow in Medicine

local lesions, or the development of a generalized roseola in a case of primary syphilis All of these signs and symptoms occur between 4 and 48 hours after the initial injection of penicillin.

The available evidence suggests that these febrile reactions occur only in syphilis and yaws They have not been observed after the administration of penicillin in patients with non-spirochetal infections

A detailed evaluation of the febrile phase of this reaction is of practical significance in relation to its possible use as an aid in the diagnosis of undetected early syphilis Also studies of the reaction in relation to initial dosage have an indirect application to the problem in neurosyphilis and cardiovascular syphilis of finding a method of preventing symptomatic reactions The studies to be presented are also of theoretic interest in relation to the mechanism of this unusual reaction

RESULTS

1 INCIDENCE The incidence of febrile reactions following the administration of crystalline penicillin G was studied among a group of 939 patients with early infectious syphilis treated by 20 cooperating clinics These patients were treated with initial doses of 25 000 to 50 000 Oxford units and with total doses of 2 400 000 to 4 800 000 Oxford units of penicillin administered intramuscularly Temperatures were recorded every four hours

Of this group 384, (41 per cent) had febrile reactions (Table

TABLE I

The Relationship of the Incidence of Febrile Reactions to the Race and Sex of Patients With Early Syphilis Following Crystalline Penicillin G

Race	Total No Treated	No with Febrile Reactions	Percentage with Febrile Reactions
White	186	85	46
Negro	753	299	40
Total	939	384	41
Sex			
Male	498	207	42
Female	441	177	40
Total	939	384	41

No significant race or sex differences in the incidence and verity of febrile reactions were demonstrated

2 RELATIONSHIP TO THE STAGE OF EARLY SYPHILIS The relaonship of the incidence and severity of febrile reactions to the age of early syphilis among 800 patients treated with crystal ne penicillin G is shown in Table 2 Febrile reactions were bserved with equal frequency and severity in patients with ronegative primary, seropositive primary, and secondary yphilis The incidence of this phenomenon was 39 to 43 per nt in these three groups of cases The height of the febrile esponse was just as great in the group of patients with seroegative primary syphilis as it was in the groups of patients ith seropositive primary and secondary syphilis

TABLE 2

The Relationship of the Incidence and Severity of Febrile Reactions to the Stage of Early Syphilis in Patients given Crystalline Penicillin G

Type of Febrile Reaction	Seronegative Primary Syphilis		Seropositive Primary Syphilis		Secondary Syphilis		Total	
	Number Treated	%	Number Treated	%	Number Treated	%	Number Treated	%
Fever All Grades	46	*39*	92	*43*	195	*42*	333	*42*
Grade I (0 to 1°F rise)	23	*19*	36	*17*	113	*24*	172	*22*
Grade II (1 to 2°F rise)	9	*8*	23	*11*	49	*11*	81	*10*
Grade III (2 to 7 F rise)	14	*12*	33	*15*	33	*7*	80	*10*
No febrile Reaction	72	*61*	124	*57*	272	*58*	467	*58*
Total	118	*100*	216	*100*	462	*100*	800	*100*

3 RELATIONSHIP TO DOSAGE A group of 121 patients with early infectious syphilis was studied at the Johns Hopkins Hospital In each case the diagnosis was established by the demontration of *Treponema pallidum* Those with fever prior to treatment were excluded from the study The initial doses administered intramuscularly to those patients varied from 1 to 120 000 Oxford units of penicillin per kilogram of body weight In these

patients who received more than a single dose within the first 24 hours, the amount given during the first six hours of treatment was arbitrarily chosen as that amount responsible for the Herxheimer reaction (Table 3). Temperatures were recorded rectally every two hours, and a rise above 100°F. (37.8°C.) was considered significant.

The incidence of febrile reactions over a wide dosage range (10 to 120,000 u./kg. of penicillin varied from 40 to 56 per cent with no definite trend in relation to dosage. However, when doses of penicillin of less than 10 Oxford units per kilogram were given, febrile reactions were no longer observed.

TABLE 3

The Relationship of the Incidence of Febrile Reactions to the Dosage of Crystalline Penicillin G Administered to 121 Patients With Early Syphilis

Penicillin Dosage Given in First 6 hours		Number of Patients	Number With Febrile Reactions	Percentage With Febrile Reactions
mg./kg.	units/kg.			
13–80[a]	20 000–120 000	9	5	56
2.6[b]	4 000	34	16	47
1.3[b]	2 000	22	12	55
0.038[c] 0.075	57 112	18	8	44
0.013[a]	20	10	4	40
0.007[a]	10	10	4	40
0.003[a]	5	8	0	0
0.0007[a]	1	10	0	0

[a] Single dose only in first 48 hours

[b] Therapeutic doses repeated every two hours

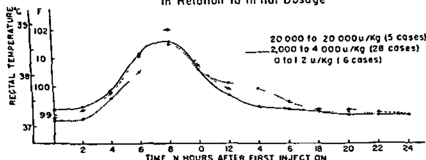
[c] Three divided doses in first six hours, and no further penicillin for 48 hours

4 PATTERN OF FEVER The pattern of the febrile response was studied in the group of 49 patients who had shown Herxheimer reactions (Table 3) Since temperatures had been recorded every two hours in this group detailed observations of the time of onset, height and duration of fever were available These factors were analyzed in the following three groups of cases 5 cases, which received large initial doses, 28 cases which received average therapeutic doses and 16 cases which received small doses of penicillin

The temperature curves of 28 patients with febrile reactions in response to average therapeutic doses of penicillin (2000 to 4000 u /kg) were studied The average temperature curve of this group is shown in Figure 1 The febrile responses followed

FIG 1

Average Febrile Reactions
Following the Use of Crystalline Penicillin G in Early Syphilis
in Relation to Initial Dosage



a uniform temporal pattern with the height of the fever as the chief variable The earliest elevation of temperature occurred four hours after injection By six hours 72 per cent of the group had febrile rises above 100°F (37 8°C), and at eight hours, 90 per cent of them showed a rise in temperature The maximal elevations were recorded between six and ten hours after injection in 90 per cent of the group In two cases the maximal febrile responses occurred between 12 and 16 hours after injection All of the temperature recordings were normal within 24 hours after the first injection

The average temperature curves of 5 patients with febrile reactions following large initial doses (20 000 to 120 000 u /kg) and of 16 patients given small initial doses (10 to 112 u /kg) of penicillin are presented in Figure 1 for comparison with the group given therapeutic doses Over this wide dosage range the pattern of the febrile reaction did not change The earliest onset

of fever was 4 to 6 hours after injection with the largest as well as with the smallest doses of penicillin which produced a reaction The maximal elevations of temperature were recorded between 6 and 10 hours after injection in each of these groups, and the *average heights of the fever (101 5 to 102°F) (38 7° to 38 9°C)* were approximately the same All of the temperature recordings were normal within 24 hours after the first injection over this dosage range The individual maximal temperature recorded in the large initial dosage range was 103 4° (39 7°C) at 8 hours after injection, in the therapeutic dosage range, 105°F (40 6°C) at 6 hours after injection, and in the small dosage range (104 2°C) at 6 hours after injection

FIG 2a

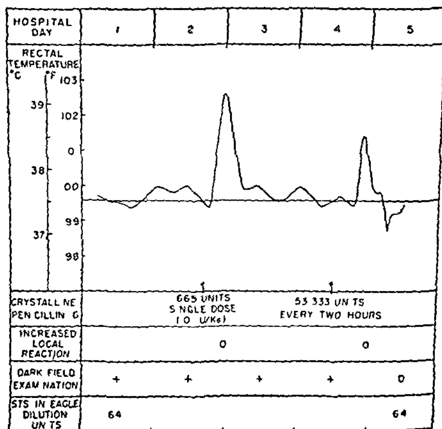
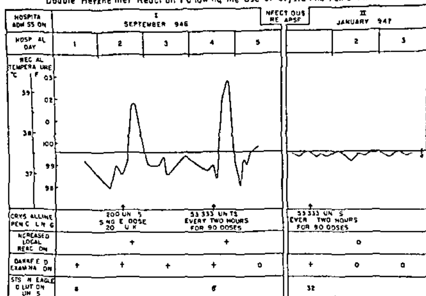


Fig. 2b

Double Herxheimer Reaction Following the Use of Crystalline Penicillin G



The similarity of the pattern of fever in response to small doses and also to therapeutic doses of penicillin is further illustrated in Figures 2a and 2b

5 Relationship to Disappearance of Treponema Pallidum from Lesions Observations were made on the relationship of the Herxheimer reaction not only to the dosage of penicillin administered but also to the disappearance of *Treponema pallidum* from darkfield positive lesions in the same group of patients With 300 to 120,000 Oxford units of penicillin per kilogram early lesions became darkfield negative between 9 and 13 hours after the initial injections in all cases At 6 to 10 hours after injection, febrile reactions occurred in one-half of these patients With 100 to 150 Oxford units of penicillin per kilogram, lesions became darkfield negative after longer time intervals, which varied from 18 to 48 hours after injection In a low dosage range (10 to 80 u /k g), dark field examinations remained persistently positive for 48 hours However, Herxheimer reactions occurred after the same time interval and in the same proportion as was observed with larger doses Only with minute doses of penicillin (1 to 5 u /kg) were febrile reactions not observed Thus Herxheimer reactions occurred with doses of penicillin as little as one-tenth of the minimal amount required to render lesions darkfield negative

6 DOUBLE HERXHEIMER REACTION Eight patients, who had shown febrile reactions to small single doses (10 to 20 u /kg) of penicillin (Table 3), again developed febrile reactions when they were given either a therapeutic dose or a repeated small dose of penicillin 24 to 72 hours later This double Herxheimer reaction is illustrated in figures 2a and 2b The temporal pattern and the height of the second febrile reactions were similar to those of the first In these patients repeated darkfield examinations remained positive for *Treponema pallidum* in the time intervals between the first and second doses

This reduplication of the reaction was not observed after repeated large single doses of penicillin Five patients, who had shown febrile reactions to large initial doses (20,000 to 120,000 u /kg) of penicillin (Table 3), were given therapeutic doses (2,000 to 4,000 u /kg) of the drug 24 to 72 hours later None of these patients showed a second febrile reaction Darkfield examinations in this group of patients became negative for *Treponema pallidum* within 13 hours after the first injection

SUMMARY

1 Febrile Herxheimer reactions were observed in 41 per cent of 939 patients with early infectious syphilis treated with crystalline penicillin G No race or sex differences in the incidence of febrile reactions were noted
2 Febrile reactions were observed with equal frequency and severity in seronegative primary, seropositive primary and secondary syphilis
3 In a group of 56 patients with relapsing secondary syphilis treated with penicillin the incidence of fever was 50 per cent with the first treatment course and 38 per cent with the second course of therapy Recurent reactions were no more frequent in those whose first course of treatment was attended by a febrile reaction than in those who had had no fever the first time
4 Within a wide range of penicillin dosage (10 to 120 000 u /kg) the incidence of febrile reactions remained relatively constant (40 to 56 per cent) With extremely small doses of penicillin (1 to 5 u /kg) febrile reactions were not observed
5 The temporal pattern of the febrile reaction was quite uniform, and was independent of the dosage over a wide range
6 Febrile reactions occurred with single doses of penicillin as little as one tenth of the amount required to render early syphilitic lesions dark field negative

With repeated small doses of penicillin (10 to 20 u /kg) two febrile reactions were produced in the same patient This double Herxheimer reaction was not observed after repeated large doses of penicillin

The mechanism of the Herxheimer reaction from penicillin in early syphilis is not clear It is suggested that the available evidence does not justify the hypothesis that the reaction is due solely to the sudden destruction of large numbers of spirochetes with the liberation of split proteins or endotoxins Further studies of the phenomenon are desirable

MORPHOLOGIC CHANGES IN SYPHILITIC LESIONS DURING THE HERXHEIMER REACTION

By

Walter H Sheldon and *Albert Heyman**

The Jarisch Herxheimer reaction has long been recognized a complication of the treatment of syphilis This reaction an unique phenomenon since it occurs only in response to itisyphilitic therapy It is thought to be caused by the release spirochetal breakdown products The syphilitic lesions frequently show gross changes during this reaction but no histogic observations have been reported

We have made histologic studies of the cutaneous and mucosal sions during the Herxheimer reaction in several groups of atients with secondary syphilis Definite histologic changes cur during this reaction These consist of congestion, edema, lteration of the vascular endothelium and acute inflammatory ll infiltration The changes are confined strictly to the syphitic lesions They appear within 5 hours after treatment and ubside within 18 24 hours These histologic changes were found i practically *all patients with clinical Herxheimer reaction in* ite syphilitic lesions of the cardiovascular and central nervous ystem

Our findings show that histologic changes occur in syphilitic sions during the Herxheimer reaction These changes may ccount for the serious clinical complications which are occaionally encountered Our findings also reveal a similarity be ween the morphologic changes of the Herxheimer reaction and he tuberculin type of hypersensitivity Further studies are in rogress which may lead to a better understanding of some of he immunologic aspects of syphilitic infection

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* From the Departments of Pathology and Medicine of Grady Memoal Hospital and Emory University School of Medicine Atlanta Georgia

THE JARISCH-HERXHEIMER REACTION IN NEUROSYPHILIS

By
*Mark T Hoekenga** and *Thomas W Farmer***

This study of the Jarisch Herxheimer reaction is based upon an investigation of 349 afebrile patients with neurosyphilis who were treated with aqueous penicillin alone at the John Hopkins Hospital between 1944 and 1948 It is concerned primarily with febrile reactions and only secondarily with symptomatic phenomena No attempt is made to compare these reactions with those observed after other methods of treatment Rectal temperatures were recorded every two to four hours on all patients and significant elevation of temperature was arbitrarily defined as a rise above 100° F (37 8° C)

Results

1 INCIDENCE OF FEBRILE REACTIONS The total incidence was 34 per cent Reactions occurred after commercial penicillin in 35 per cent (76 of 216 patients) and after crystalline penicillin

TABLE I
The Relationship of the Incidence of Febrile Reactions to the Race and Sex of Patients with Neurosyphilis Following the Administration of Penicillin

	Number Treated	Febrile Reactions	
		Number	Percentage
RACE			
White	115	35	30
Negro	234	84	36
SEX			
Male	235	82	35
Female	114	37	32
Total	349	119	34

G† in 32 per cent (43 of 133 patients) Since incidence was not related to type of penicillin the two groups are combined in all analyses except those relating to dosage

No significant race or sex differences were demonstrated (Table I)

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** The Johns Hopkins University and the United States Public Health Service Venereal Disease Research and Post-Graduate Training Center and Senior Fellow in Medicine National Institute of Health

† The crystalline penicillin G used in these studies was supplied by the Commercial Solvents Company lot numbers 46042605 and 4608?605 Potency 1500 1540 units per milligram

2 INCIDENCE OF SYMPTOMATIC REACTIONS Among the 349 ases in this series, only 6 (1 7 per cent) had aggravations of nental or neurologic symptoms during the first one to six days fter onset of treatment There were convulsions in two intances, confusion and disorientation in two, hallucinations and elusions in one and signs of meningeal irritation in one All six f these patients had associated fever In four of them, the liagnosis was dementia paralytica (an incidence of 7 3 per cent or this diagnostic group) and in two, asymptomatic neuroyphilis (rate of 1 4 per cent) The two individuals with asympomatic neurosyphilis and one of those with dementia paralytica iad only transient symptoms with no recurrence during the next wo to three years The other three patients, who had been rec-

TABLE II

The Relationsh p of the Inc dence of Febr le React ons to the Stage and Type of Neurosyphil s

D agnost c Type	Stage	Number Treated	Febr le React ons	
			Number	Percentage
Acute syph l t c men ngitis	Early	17	4	24
Asymptomat c	Early & Late	136	33	24
Men ngovascular	Early & Late	78	28	36
Dement a para yt ca and Tabopares s	Late	55	41	74
Tabes dorsal s with or without pr mary opt c atrophy	Late	40	9	23
Other types	Late	23	4	17
Total		349	119	34

ognized as advanced cases of dementia paralytica before treatment was commenced, were committed to mental institutions, one of these was probated after three months, but the other two have required custodial care for periods of nine and thirty months respectively

3 RELATIONSHIP TO THE DURATION OF SYPHILITIC INFECTION AND TO THE DIAGNOSTIC TYPE OF NEUROSYPHILIS In Table II it is seen that among 17 patients with early acute syphilitic meningitis, only 4 (24 per cent) had febrile reactions to penicillin Similarly in 27 cases of early asymptomatic neurosyphilis or meningovascular neurosyphilis, 7 (26 per cent) had such re sponses In late neurosyphilis, exclusive of dementia paralytica, this phenomenon occurred in 24 to 36 per cent of the individuals treated However, it was observed in 41 (74 per cent) of 55

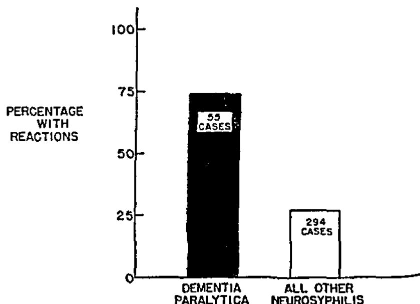


FIG 1

RELATIONSHIP OF THE INCIDENCE OF EARLY FEBRILE REACTIONS TO THE DIAGNOSTIC TYPE OF NEUROSYPHILIS

patients with the diagnosis of dementia paralytica (Table II and Fig 1).

4 RELATIONSHIP TO CEREBROSPINAL FLUID FINDINGS For the purpose of this study, we have arbitrarily regarded a white cell count of 10 or more or a protein content of 40 mgm% or more in the cerebrospinal fluid as abnormal Complement fixation titers of 0 2 to 1 0 cc have been defined as moderately positive, those of 0 01 to 0 1 cc, as strongly positive

The results presented in Table III and in Figure 2 show that the frequency of reactions increased directly with elevations in white cell count and total protein, irrespective of the titer of the complement fixation test This increase was more marked in patients with dementia paralytica but was also seen to a lesser extent in patients with other types of neurosyphilis The highest incidence (93 per cent) was observed in patients with dementia paralytica and with both cell count and protein content abnormal

When incidence was related to the titer of the reaction in the complement fixation test, to the exclusion of elevated cells

TABLE III

Relationship of the Incidence of Febrile Reactions to the Abnormalities of the White Cell Count and the Protein Content of the Cerebrospinal Fluid

White Cell Count and Protein Content	Dementia Paralytica			Other Neurosyphilis			Total		
	No Cases	Febrile Reactions		No Cases	Febrile Reactions		No Cases	Febrile Reactions	
		Number	Per Cent		Number	Per Cent		Number	Per Cent
Both normal	7	1	14	46	5	10	53	6	11
Cells or protein elevated	17	11	64	87	17	19	104	28	26
Both elevated	31	29	93	161	56	34	192	85	44

TABLE IV

Relationship of the Incidence of Febrile Reactions to the Complement Fixation Titer of the Cerebrospinal Fluid

Complement Fixation	Dementia Paralytica			Other Neurosyphilis			Total		
	No Cases	Febrile Reactions		No Cases	Febrile Reactions		No Cases	Febrile Reactions	
		Number	Per Cent		Number	Per Cent		Number	Per Cent
Negative or moderately positive	6	1	16	114	15	13	120	16	13
Strongly positive	49	40	81	180	63	35	229	103	42

TABLE V

The Relationship of the Incidence of Febrile Reactions to Cerebrospinal Fluid Abnormalities

Cerebrospinal Fluid Findings		Dementia Paralytica			Other Neurosyphilis			Total		
Complement Fixation	White Cell Count and Protein Content	No. Cases	Febrile Reactions		No Cases	Febrile Reactions		No Cases	Febrile Reactions	
			Number	Per Cent		Number	Per Cent		Number	Per Cent
Negative	Both normal	0	0	0	10	1	10	10	1	10
Moderately Positive	Both normal	2	0	0	32	3	9	34	3	9
	Cells or Protein elevated	3	0	0	43	6	14	46	6	13
	Both elevated	1	1		29	5	17	30	6	20
Strongly Positive	Both normal	5	1	20	4	1	25	9	2	22
	Cells or Protein elevated	14	11	79	44	11	25	58	22	38
	Both elevated	30	28	93	132	51	39	162	79	49
Total		55	41	74	294	78	27	349	119	34

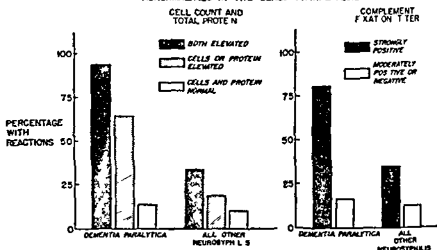


FIG 2
RELATIONSHIP OF THE INCIDENCE OF FEBRILE REACTIONS TO THE ABNORMALITIES IN THE CEREBROSPINAL FLUID

and proteın ıt was observed that the more strongly posıtive the test the more frequent were febrıle reactıons This was true ın all types of neurosyphılıs but especially ın patıents wıth dementıa paralytıca (Table IV and Fıg 2)

A finer breakdown of the data ıs provıded in Table V

5 RELATIONSHIP TO BLOOD SEROLOGIC TESTS FOR SYPHILIS There was no correlatıon of the ıncidence of febrıle reactıons with serologıc negativıty or with the heıght of the serologıc tıter

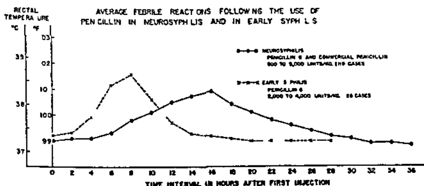
6 RELATIONSHIP TO PREVIOUS TREATMENT Among 107 patıents never previously treated febrıle reactıons occurred ın 41 (38 per cent) Of 87 patıents who had receıved metal chemotherapy wıthın a year of the admınıstratıon of penıcıllın 28 (32 per cent) had febrıle reactıons Of 14 patıents who had receıved therapeutıc malarıa from two months to several years before admınıstratıon of penıcıllın only 1 (7 per cent) had a febrıle rıse In 9 patıents who had receıved courses of penıcıllın 2 months to 2 years before present treatment 2 (22 per cent) had reactıons

7 RELATIONSHIP TO DOSAGE To 202 patıents ınıtıal doses of penıcıllın G rangıng from 10 unıts to 20 000 unıts per kılogram of body weıght were admınıstered (Table VI) In each of the dosage groups the percentage of patıents wıth markedly abnormal cerebrospınal fluıd findıngs and/or dementıa paralytıca was approxımately the same The occurrence of febrıle reactıons over thıs wıde dosage range varıed only from 26 to 32 per cent

with no definite trend except that when doses as small as 10 units per kilogram were given temperature responses were no longer observed

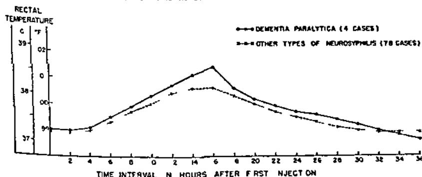
8 PATTERN OF FEVER The pattern of the febrile response was studied among the 119 patients described in Table II The average temperature curve of this group is shown in Figure 3, it is really composed of reactions which were maximal at intervals varying all the way from 6 to 24 hours after the injection of penicillin This is in marked contrast with the uniform pat-

FIG 3



tern observed in early syphilis The earliest elevation of temperature occurred four hours after injection Between 12 and 16 hours, 50 per cent of the maximal responses occurred, and the remaining cases had equal distribution before or after this middle period Approximately 75 per cent of the patients had temperatures above 100° F (37 8° C) at 16 hours after the injection of penicillin

FIG 4



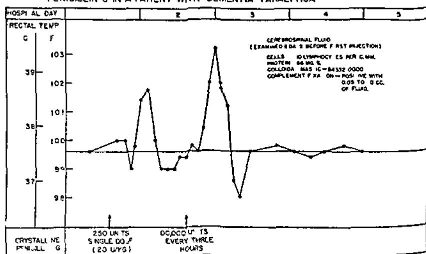
There was also considerable variation in the duration of fever, but all of the temperature recordings were normal within 36 hours after the first injections

The patterns of febrile response were similar in all the diagnostic types of neurosyphilis The average temperature curve of the patients with dementia paralytica is presented in Figure 4 for comparison with that of all other types of neurosyphilis The maximal responses in both groups were reached in 16 hours after injection

The pattern of fever in 43 patients given usual therapeutic doses of crystalline penicillin G was approximately the same as that observed in 12 patients given small initial doses (20 to 40 u /K) The earliest onset of fever was 4 hours after injection in each of the two groups The individual maximal elevations of temperature were 103 2° F (39 6° C) and 103 8° F (39 9° C) in these two groups This similarity in response to small doses and also to therapeutic doses in the same patient is illustrated in Figure 5A

FIG 5A

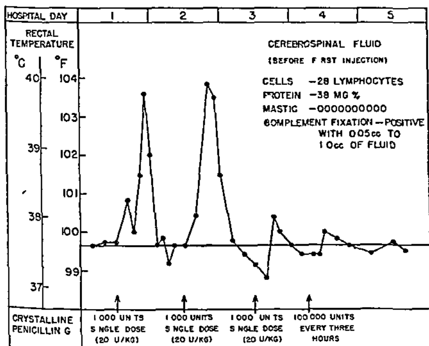
DOUBLE FEBRILE REACTION FOLLOWING THE USE OF CRYSTALLINE PENICILLIN G IN A PATIENT WITH DEMENTIA PARALYTICA



9 DOUBLE HERXHEIMER REACTIONS Of the 12 patients who had shown febrile reactions to small single doses of penicillin (Table VI), 9 again developed fever when they were given either a therapeutic dose or a repeated small dose of penicillin 24 to 72 hours later This double Herxheimer reaction is illustrated in Figures 5A and 5B Repetition of fever was not observed after repeated large single doses of penicillin

Fig 5B

MULTIPLE FEBRILE REACTIONS FOLLOWING THE USE OF CRYSTALLINE PENICILLIN G IN A PATIENT WITH LATE ASYMPTOMATIC NEUROSYPHILIS



Summary

1 Febrile Herxheimer reactions were observed in 34 per cent of 349 patients with various types of neurosyphilis treated with penicillin 74 per cent of patients with dementia paralytica had reactions The incidence of reactions was also significantly higher in patients with abnormal cerebrospinal fluid findings

2 The incidence of the reactions in patients recently treated with metal chemotherapy was similar to that observed in previously untreated patients

3 The temporal patterns of the febrile reactions in neurosyphilis were quite variable Maximal febrile responses occurred over the wide range of 4 to 24 hours after the injection of penicillin Approximately 50 per cent of the patients had maximal elevations in temperature between 12 and 16 hours after injection

4 The incidence of febrile reactions following small initial doses of penicillin (20 to 40 units/K) was the same as that observed after the usual therapeutic doses With extremely small

amounts (10 u /kg) of penicillin, no temperature elevations occurred

5 With repeated small doses of penicillin (20 u /kg), double and multiple febrile reactions were produced in the same patient

6 These observations demonstrate that febrile reactions in neurosyphilis cannot be prevented by the administration of small initial doses of penicillin in the range of 20 u /kg

DISCUSSION OF PAPERS ON THE JARISCH HERXHEIMER REACTION

J Lamar Callaway, (Duke Hospital, Durham, N C) Our experience at Duke Hospital parallels the experience of investigators at John Hopkins Hospital concerning some of the severe Herxheimer reactions in syphilis of the central nervous system We have seen febrile Herxheimer reaction in patients with central nervous system syphilis following intramuscular injection of 500 units of crystalline G" penicillin

Some of our most severe clinical Herxheimer reactions have occurred in patients with "asymptomatic neurosyphilis" who had a grade 3 type of spinal fluid It is entirely possible that if these patients had had careful psychometric examinations they would have been classified not as asymptomatic neurosyphilis but as pre paresis or early paresis It seems desirable that such psychometric examinations be done on patients if possible

We have had both 'asymptomatic neurosyphilis' patients and patients with general paresis who have had mild to normal electroencephalograms before penicillin was started develop severe abnormalities of the electroencephalograms after clinical Herxheimer reaction

We have also observed patients who develop a mild febrile Herxheimer reaction on small doses of penicillin in whom the drug was continued in a gradual step up fashion only to have them show a more severe febrile Herxheimer reaction with clinical symptoms when the full dosage was reached This may represent a double Herxheimer reaction

Dr Moore has pointed out an interesting observation in which it appears that patients may do better if they are started on fever therapy before penicillin rather than giving the penicillin followed by fever therapy Except for the investigative aspects it would seem desirable to give patients a course of bismuth before fever and penicillin therapy is instituted

PENICILLIN IN THE TREATMENT OF UNCOMPLICATED GONORRHOEA

By

*Ambrose King**

There is no doubt that penicillin is a major advance in the methods of treatment of uncomplicated gonorrhoea if judged only by the prompt effect in limiting spread of infection and in preventing complications The beneficial results have been so obvious and so striking as to convince many that gonorrhoea is no longer a public health problem, and that it may be disregarded in any programme of research I propose to bring evidence to suggest that some successes of treatment with penicillin are more apparent than real, and that gonorrhoae retains its character as a disease which tends to infectious latency In the reported cases the evidence of clinical cure is usually supported by brief observation, lasting a few days or a few weeks, and perhaps by a few negative smears and cultures from the prostatic secretion or from the urethra and cervix I contend that such criteria of cure are unreliable My doubts are based on clinical observation which is even more important than microscopic and cultural tests which, like all other tests suffer from inherent difficulties of technique A positive culture test from a reliable laboratory cannot be ignored, but a negative test from the same source may well be of no significance In nearly all the male patients the gross signs of urethritis clear very quickly, and if the patient is examined during the day, after holding urine for two or three hours, he seems quite well and free from signs If, however, he attends in the early morning before passing urine, and having held it for eight hours or more, there are, in many cases, signs of residual infection such as slight secretion at the meatus from which smears show polymorphonuclear leucocytes, and in an occasional case, gonococci, and the urine shows pus or pus threads These signs often persist, and there may be also excess of polymorphonuclear leucocytes in the prostatic secretion Some of these cases relapse with purulent urethritis, and gonococci are found again Such relapses are usually attributed to re infection

In most female patients treated with penicillin the organisms rapidly disappear from the secretions, and any symptoms due to gonorrhoea are relieved Signs of infection of the cervix are,

* Whitechapel Venereal Disease Clinic London England

however, very apt to persist, and erosion and mucopurulent discharge may remain indefinitely. In some cases the gonococcus persists or re appears, and in an occasional case it continues to do so in spite of massive doses of penicillin

The question of relapse or re infection can never be settled finally because the two conditions are indistinguishable I suggest, therefore, that in assessing results the evidence should be weighted against the drug under investigation, and that all cases in which gonococci are found within three months of the time of treatment should be regarded as "relapses" This would be an important step to bring these investigations in line with the much more careful and conservative studies which have been made on the effect of penicillin in the treatment of early syphilis The explanation which is sometimes advanced to explain the re-appearance of gonococci is that there are foci of infection to which the penicillin in the blood stream does not penetrate There is little or no evidence for or against an hypothesis of this kind for in most cases these patients show no clinical evidence of abscess formation or any other complication

In assessing these cases it may be demanded as the ultimate criterion of "penicillin resistance" that gonococci in subculture should be proved to be resistant to penicillin in vitro Undoubtedly this test should be done, but, for various technical reasons concerned with the difficulty of isolating the organism in pure culture, we have not yet succeeded in applying the test to our own cases as a routine procedure At the same time it may be questioned whether the reaction of the gonococcus in the test tube are a real index of its probable behaviour in vivo The incalculable factor of tissue immunity in the host remains In the days when gonococci were termed "sulphonamide resistant" there was little correlation between the findings in vivo and vitro

To support my case for the validity of these views I present the details of analysis of 1555 cases of fresh, acute, uncomplicated gonorrhea in the male and 219 cases of similar infection in the female All the female patients and 693 of the male patients were infected for the first time All were treated with a routine course of 150,000 units of commercial sodium penicillin in water, consisting of five doses of 30,000 units given at intervals of two hours

Of the 1555 men, 283 did not attend for observation, and nothing is known of their subsequent history, leaving 1272 in whom some assessment could be attempted Three hundred and fifty

even of these attended for three months or more and 915 for less han three months Eighty two (6 5 per cent) must be classed s early failures because gross signs of infection persisted or reappeared within two weeks Of these 44 showed signs of urethritis, with gonococci persisting in 23, while 26 had gross pyuria without urethral discharge Such failures responded, for the most part, to further courses of penicillin Five other patients developed epididymitis, five arthritis and two Cowperitis within the first week after treatment with penicillin During observation after apparent cure from two to thirteen weeks after treatment no less than 242 (19 per cent) of these patients developed an acute purulent urethitis, and 197 (15 per cent) of them showed *gonococci in the secretions Of the 197,* ninety-seven admitted further sexual intercourse, one hundred of them did not In addition 214 (17 per cent) symptomless patients showed evidence of residual infection in the prostate or seminal vesicles twenty four of them with abnormalities of prostate or seminal vesicles detected by the examining finger and 166 showing gross excess of leucocytes in the prostatic secretion, five others showed gonococci in prostatic smear and nineteen

TABLE I

Males	Observation		Early Failures	Relapses	Residual Infection	Total Late Failures
	3 months or more	Less than 3 months				
1555 less 283 = 1272	357	915	82 = 6 5%	242 = 19% Gc+ = 197 (15%) Gc— = 45	214 = 17% or 99 = 8%	4 6 = 36% or 341 = 27%

gave positive cultures of the vesiculo prostatic secretion It may be contended that in some cases such residual findings were due to past attack of infction One hundred and fifteen of these 214 patients gave histories of previous infection and 99 did not Accepting only the 99 as proved failures in this sense, the proportion is reduced to 8 per cent The total late failures obtained by adding 'relapses" to those with residual infection amounts to 456 (36 per cent) or, after correction, 341 (27 per cent)

Of two hundred and nineteen women twenty did not attend for observation and therefore assessment was possible in only

one hundred and ninety-nine One hundred and fifteen of these remained under observation for three months or more, and eighty-four for less than three months As I have indicated most of these women continued to show some signs of infection, and immediate failure was assessed in terms of the continued presence of the gonococcus or its reappearance within two weeks This happened in six cases (3 per cent). Disappearance of gonococci followed re-treatment with penicillin In 32 (16 per cent

TABLE II

Females	Observation		Early Failures	Relapses	Residual Infection
	3 months or more	Less than 3 months			
219 less 20 = 199	115	84	6 = 3%	32 Gc+ = 16%	Most showed s gns

of these patients the gonococcus reappeared during the late period of observation—in 17 during the first month, five during the second month, four during the third month, and in 6 more subsequently Twelve admitted sexual intercourse and twenty denied it Among those who relapsed during the months of observation six developed the signs of salpingitis and one Bartholinitis

This group of cases, male and female therefore showed an immediate response to penicillin which was just as satisfactory as the general experience led us to expect There was, notwithstanding, much evidence to suggest that in a considerable number the infection was suppressed rather than cured The continuing evidence of a persisting low-grade infection, combined with clinical relapse in a high proportion is, to my mind, strong evidence that the results with this treatment are less satisfactory than is generally believed I am aware that many would be prepared to write off the cases I have described as "relapses" as "reinfections" Yet the coincidence that such a large number of patients in this group, 197 men and 32 women, developed a further attack of gonorrhoea within a few months is, to my mind too great to be convincing The patients who continue observation for this period are, in general, the more balanced and less promiscuous group The coincidence is even more remarkable when one considers that 58 of the 197 men and all 32 women had

been treated for first attacks of gonorrhoea It seems unlikely that they would be unfortunate again so soon It is always necessary to remember, too, that one of the most sensitive provocative tests for latent infection is the resumption of sexual intercourse

I appeal then for more emphasis to be placed upon clinical observation in tests for cure Judged by this standard alone there is an awakening ahead for those who so firmly proclaim that gonorrhoea is no longer a problem The value of cultural tests has been stressed in recent years and certainly they are the most important of the accessory methods available, but the sensitivity of these tests is subject to great variation in different laboratories, and even in the same laboratory at different times Without clinical observation they are an unreliable index of cure I appeal, also, for a more objective approach to the question of "relapse" occurring within three months of apparent cure Bearing in mind that this often happens to those patients who are known to be reliable, and that sexual intercourse is itself an excellent provocative test I believe that all such cases should be classed as "relapses," whether the possibility of reinfection is admitted or not

VIABILITY STUDIES WITH *N. GONORRHOEAE*

By

Justina H. Hill, E Ellen Nell, and *Adelaide M Mueller**

We wish to report today experiments on the influence of certain chemical factors upon, first, the growth of the gonococcus, and, second, upon its viability

The experiments upon the growth of the organism deal chiefly with the effect of certain amino acids and vitamins These have been studied from two points of view, that is, first, their possible stimulating or toxic action in an adequate medium, and, second their possible utilization by the gonococcus in an inadequate base

The adequate medium is that of Gould, Kane and Mueller[1], except that it has been further simplified by the omission of histidine, because, as we showed last year, we have not found this amino acid of value in this medium The inadequate medium, or base, which does not support the growth of the gonococcus is the complete medium from which the glutamic acid has been omitted You will note that the only sources of nitrogen in the complete medium are the glutathione and the glutamic acid, and that the only sources of carbon are the glutathione, glutamic acid, glucose and starch To these one must add the carbon dioxide in which the cultures are incubated

In regard to nitrogen metabolism, the evidence is that it is the glutamic acid which must serve as the source of this element Although glutathione is one of the most important ingredients of the medium, the evidence now is that its value is in the reversible oxidation reduction system provided by its sulfhydryl group The fact that no strain of gonococcus will grow in the base, in which glutathione is the only source of nitrogen, supports the fact that glutathione is not itself used directly by this organism for nutrition

This same fact makes it clear that the gonococcus does not use glutathione as a source of carbon There is no evidence that the gonococcus can attack starch Many strains grow without glucose No evidence has yet been obtained that the gonococcus can utilize atmospheric carbon dioxide under the conditions of these experiments One is therefore justified in concluding that the glutamic acid is the source, not only of nitrogen, but also of carbon in the adequate medium

In considering the effect of the amino acids, it is impossible to correlate the previous investigations in which a fairly wide

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range of these substances has been studied Thus McLeod, Wheately and Phelon[2], Lankford, Scott, Cox and Cooke[3], Landy and Gerstung[4] and Gould[5] all used media which contained either peptone or protein hydrolysates, which were not analyzed for their amino acid contents In view of the interaction of many of the amino acids, as, for example, between those that can serve as hydrogen donators and those that can act as hydrogen acceptors, and of the variation of the significance of various vitamins in relation to amino acid content, it is impossible to draw more than indications from such studies Although Hosoya and Kurnoya[6] used a synthetic base, the number of their observations was quite limited We are left, therefore, with only the previous study of Plack, Stokinger and Carpenter[7] for any general study of the effect of amino acids upon the gonococcus in a synthetic medium The complexity of their medium means that many months would be required to break it down to determine the exact role of any single ingredient or of any combination of them We have therefore, preferred to use the much simpler media we have just described and to build up, first with single amino acids, and, later, with combinations of them

We have quantitated these experiments by using, whenever solubility permitted, 5, 50 and 500 mgs per cent of the amino acid being tested It should also be stated here that marked differences are observed with different strains, so that it is necessary to test a number of cultures, as well as enough lots of a given medium to control any possible variations from this source

Time does not permit a detailed discussion of methods, but these may be summarized by stating that 18 hour cultures of recently isolated strains are suspended and washed three times in buffered saline, pH 7 2 7 4, containing 0 15 per cent soluble starch Plates are inoculated with 0 05 ml of final suspension, streaked, and incubated 48 hours at 36° C in 10 per cent carbon dioxide They are then examined grossly and under a wide field microscope, and tested for the oxidase reaction Smears and subcultures are made when indicated

The effect of the inclusion of certain single amino acids upon growth in the adequate medium is shown in Table 1 Alanine, leucine, tyrosine, cysteine and asparagine in appropriate amounts stimulated growth No stimulation was observed with glycine, threonine, valine, phenylalanine, cystine, methionine or tryptophane The only analysis of these findings that can be made at this time is the observation that of the three sulfur-containing

TABLE 1

Effect of Single Amino Acids upon Growth of the Gonococcus in an Adequate Medium

Amino Acid	Mg% Stimulating	Mg% No Effect	Mg% Toxic
Alanine	5 and 50	500	——
Glycine	——	5	25, 500
Leucine	5 and 50	500	——
Threonine	——	5 and 50	500
Valine	——	——	5, 500
Phenylalanine	——	25 and 50	500
Tyrosine	0.625 and 6.25	62.5	——
Cysteine	2.5	6–25	——
Cystine	——	2.5	6–50
Methionine	——	0.001–10	——
Tryptophane	——	10 and 25	500
Asparagine	5, 50 and 500	——	——

amino acids, cysteine, cystine and methionine, stimulation was obtained only with cysteine. It should also be noted that the higher concentrations of glycine, threonine, valine, phenylalanine, cystine and tryptophane were toxic.

The more important problem, the investigation of amino acids capable of permitting growth of the gonococcus in an inadequate medium is exemplified in Table 2 in regard to single amino acids or their amides. You will observe that, in comparison with glutamic acid, which in a concentration of 250 mgs. per cent per

TABLE 2

Effect of Single Amino Acids or Their Amides on Growth of the Gonococcus in an Inadequate Medium

Acid	Mg %	Total No. of Strains	No. of Strains Growing
Alanine	500	21	11
	50	21	6
Theronine	50	7	3
	5	7	4
Proline	500	8	3
	50	8	3
Asparagine	500	15	5
	50	21	5
Glutamic Acid	250	37	31
Glutamine	2	16	15
	1	16	14
	0.5	16	10

mits the growth of 31 of 37 strains, or of its amide, glutamine, which, in a concentration of 2 mgs per cent, permits the growth of 15 of the 16 strains, no single amino acid has yet been found of the same order of activity Some effects have been noted with alanine, threonine, proline and asparagine The single amino acids we have tested so far without any evidence of utilization are listed in Table 3

TABLE 3

Amino Acids Without Effect on Growth of the Gonococcus in an Inadequate Medium

Glycine
Leucine
Valine
Phenylalanine
Tyrosine
Cystine
Cysteine
Methionine
Tryptophane

In beginning to build up combinations of amino acids, we can report today those shown in Table 4 You will see that the combination of 25 mgs per cent of glycine and of the same amount of threonine has permitted the growth of all of the six strains tested, *and that the other combinations have permitted growth* of half or more of the strains tested In regard to the glycine-

TABLE 4

Effect of Combined Amino Acids on Growth of the Gonococcus In an Adequate Medium

Amino Acids	Mg %	Total No of Strains	No of Strains Growing
Glycine Threonine	25 25	6	6
Glycine Threonine Phenylalanine	25 25 25	8	5
Glycine Cystine	25 5	8	4
Glycine Tryptophane	25 25	6	3
Tryptophane Phenylalanine	25 25	8	5

threonine combination it should be noted that the glycine alone was ineffective and the threonine alone had some effect, but less than that of the combined amino acids

In regard to the vitamins, neither Lankford, Scott, Cox and Cooke[3] nor Landy and Gerstung[4] have demonstrated their value in growth of the gonococcus Our experiments so far with single vitamins, as shown in Table 5, and with a combination, shown in Table 6* confirm the lack of value of the vitamins tested under the conditions of our experiments

TABLE 5

Vitamins That Give No Stimulation of Growth When Added Singly

Classification	Vitamin
B_1	Thiamine
B_2 Complex	Calcium Pantothenate Riboflavine Niacin Choline *Pyridoxine* p Aminobenzoic Acid
C	Ascorbic Acid

In conclusion of this section on growth factors, it now seems evident that study of the amino acid requirements of the gonococcus is more important than vitamin investigations Landy and Gerstung[4] have shown that the gonococcus can synthesize p aminobenzoic acid, and, pending chemical proof, it is probable that this organism must also be able to synthesize other vitamins Work in both fields is being continued

In regard to the viability of the gonococcus, Cole and Lloyd[5] suggested that the factors for growth and for viability might be different We have studied the effect of various chemical factors upon viability, chiefly by their introduction into infusion broth The method consists essentially of the inoculation of the experimental media and with subculture at 0, 1, 3, 5, 7, 14 21 or more days to the limit of viability These tests have been done at room temperature and at normal atmosphere because a secondary purpose of the study has been the improvement of

* Prepared by Dr Robert Nelson

TABLE 6

Vitamin Mixture With No Effect on Growth or Viability

Vitamin	Micrograms Percent
Thiamine	20
Ca Pantothenate	20
Riboflavine	20
Niacin	100
Choline	1
Pyridoxine	100
Inositol	100
Biotin	0 1
Pteroylglutamic Acid	0 1

transportation media The following summaries indicate the influence of certain factors

1 The percentage of strains surviving for 3 days in four media was as follows:

a 0 9 per cent sodium chloride	0
b Infusion broth	30 0%
c Infusion broth with 0 3% soluble starch	34 0%
d Infusion broth, with 10% human whole blood	68 0%

2 A comparison of from 0 075% through 40% starch, added to infusion broth has shown that the optimal concentration is 5 or 10%

3 No difference in viability has been found in a comparison of 10% whole human blood, unheated, or "chocolated," both giving viability curves which are both higher and longer than that of infusion broth

4 The substitution of 1% hemoglobin instead of 10% human whole blood gives equally good results

General Conclusions

By the findings here reported and by further studies of the same problems knowledge is gained in regard to the fundamental requirements of the gonococcus Differences between growth and viability requirements can be determined Upon the basis of such findings it should be possible to stabilize the growth of the organism *in vitro* and to obtain definition of the colony types most significant in regard to virulence Such definition, in turn, will provide a basis for controlled investigation of the immunologic, chemoprophylactic and chemotherapeutic responses of the organism

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EXPERIMENTAL GONOCOCCAL INFECTION OF THE RABBIT'S EYE

By
*C. Phillip Miller**

During the war the Subcommittee on Venereal Diseases undertook to improve the venereal disease prophylactics employed by the Armed Forces As part of this program, an attempt was made to produce in some animal a local gonococcal infection which would lend itself to the testing of a variety of prophylactic agents

Some years before, Dr. Walter D Hawk, working in my laboratory, was able to produce infection in the mouse by the intraperitoneal inoculation of mucin suspensions of a strain of gonococcus, after its virulence had been raised by repeated animal passage At the beginning of these experiments several million organisms were required to infect a mouse, but after 130 animal passages during the course of 15 months, its virulence was enhanced to a level which enabled inocula of less than 10 gonococci to produce a fatal gonococcal sepsis

This intraperitoneal infection, however, was not suitable for the testing of prophylactic agents Numerous trials to produce urethritis or vaginitis failed in all of the ordinary laboratory animals Attempts were made, therefore, to infect the conjunctiva of the rabbit It was found that experimental gonorrheal conjunctivitis could be produced if large numbers of gonococci were placed in the conjunctival sac and the lids closed and sealed to prevent their movement Large doses of atropine were administered to reduce the secretion of tears The serious disadvantage of this method, however, was the frequency of contamination by secondary invaders

The conjunctiva was, therefore, abandoned as a site of infection in favor of the anterior chamber

The rabbits were prepared by an intravenous injection of morphine or of demerol and the local application of 5 per cent cocaine to the conjunctival sac The aqueous humor was aspirated and replaced with 0 2 cc of a saline suspension of gonococci This volume represents the average capacity of the anterior chamber of the rabbit's eye

Our best results were obtained with 18 hour cultures of a strain that had been passed several times in the rabbit's eye,

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a fact which led us to the conclusion that the organisms became adapted to this particular environment. The gonococci were found to multiply and to invade the intraocular tissues, particularly the lens and ciliary body Actual multiplication of the inoculated gonococci was proved by colony counts on suspensions of aqueous humor and macerated lens and ciliary body 24 hours after infection An acute inflammation resulted

The success of the inoculation depended upon the numbers of gonococci introduced into the anterior chamber Table 1 shows that inocula of approximately 20,000,000 gonococci resulted in 93 per cent of 'takes" There was a greater number of eyes in this category because it included the controls for a series of prophylactic experiments You will see that the percentage of positive infections fell with the size of the inocula, but that infection resulted in almost half of the eyes receiving as few as 200 gonococci

TABLE 1

INFECTIONS RESULTING FROM INOCULATION OF DIFFERENT NUMBERS OF GONOCOCCI

Approximate number of gonococci	*Number of eyes inoculated*	*Number of infections resulting*	*Per cent*
20,000,000	266	248	93
2,000,000	32	27	84
200,000	28	23	82
20,000	32	21	65
2,000	26	12	46
200	22	10	45

Duration of the infection was determined by inoculating a large series of animals and keeping them under observation for periods up to 14 weeks They were killed at intervals and cultures were made of the several parts of the eye

In Table 2 you will see that after the first week approximately 1/3 of the eyes were still infected In other words, the infection became chronic in about a third of the eyes and persisted for as long as 14 weeks, the longest period of observation

TABLE 2

DURATION OF INFECTION

As determined by recovery of viable gonococci from eyes at various times after inoculation

Time after inoculation	*Number of eyes cultured*	*Number of eyes positive*	*Per cent positive*
1 day	10	10	100
2 days	11	10	91
3 "	12	9	75
4 "	12	7	58
5 "	15	7	46
6 "	24	11	45
7 "	23	8	35
2 weeks	6	2	
3 "	4	2	
4 "	4	1	
5 "	4	1	
6 "	4	2	
7 "	2	1	
8 "	6	3	
9 "	4	2	
10 "	10	3	
11 "	6	2	
12 "	2	0	
14 "	2	2	

The acute inflammatory reaction gradually subsided and many cultures of aqueous humor showed no growth but at autopsy at the end of 9 weeks, cultures of the lens showed a confluent growth of gonococcus

Agglutination and complement fixation tests were run on sera obtained at weekly intervals on all of the rabbits in which the infection was allowed to become chronic None of them developed agglutinins to a significant titre but a majority developed complement fixing antibodies indicating a humoral response to the localized infection in the eye

The complement fixation test, however, was no more indicative of existing infection than it is in man A positive test merely signified the presence of infection at some time in the past

When this experimental infection was used for the testing of prophylactic agents, the drug was introduced directly into the anterior chamber within an hour after a standard inocula of 20 000,000 gonococci, a number which produced infection in 93 per cent of the controls These eyes were enucleated after 24 hours The aqueous humor was aspirated and cultured The cornea was then removed and the anterior chamber thoroughly rinsed with sterile saline The ciliary body was macerated and cultured and the anterior surface of the lens rubbed across the surface of a blood agar plate

The most effective prophylactic was penicillin which in a dose as small as 2½ units was uniformly effective in sterilizing the anterior chamber of gonococci Doses of 1 unit sterilized 87 per cent of eyes

Mild and strong silver protein even in solutions much stronger than are ever used in man were ineffective in eliminating gonococci from the anterior chamber They never sterilized more than 50 per cent of the eyes tested

A large series of tests was run with ointments containing 15 per cent sulfathiazole and 30 per cent calomel, separately and in combination (Table 3) The sulfathiazole alone or in com

TABLE 3

EFFECTIVENESS OF PROPHYLACTIC OINTMENTS

Composition of Ointment				
Base	Sulfa thiazole	Cal omel	Eyes Sterile on culture	
aqueous	15%	30%	53/53	100%
	15	—	26/26	100
		30	27/28	96
oily	15	30	7/24	30%
	15		3/8	37
		30	1/6	17
		33	8/55	15
aqueous ointment control			9/29	31%
oily ointment control			0/24	0

bination with calomel was 100 per cent effective in sterilizing the eyes of gonococci when it was made up in an aqueous or vanishing cream base Thirty per cent calomel alone in this vanishing cream ointment was effective in 96 per cent of the tests B t when these ingredients were made up in an oily or greasy base

they succeeded in eliminating gonococci from only 15 to 37 per cent of the eyes tested

The explanation for the marked difference in the effectiveness of these gonococcidal drugs in the two kinds of ointments was found in the different behavior of the two after they were introduced into the anterior chamber The watery or vanishing cream ointments which contained small quantities of a detergent or wetting agent spread easily over all the surfaces bounding the anterior chamber, whereas, the greasy or oily ointments failed to do so even when they were uniformly distributed by gentle massage of the cornea The greasy ointment very quickly reagglomerated as you will see in the photograph showing that it never properly spread and wet the tissues of the lens and iris so that the gonococci which had already penetrated into their surfaces were not reached by the gonococcidal chemicals in the ointment

SUMMARY

It was possible to produce an experimental gonococcal infection of the rabbit's eye by inoculation of the anterior chamber The gonococci were found to multiply and invade the intraocular tissues, particularly the lens and ciliary body In approximately 1/3 of the inoculated eyes, the infection became chronic and persisted for as long as 14 weeks, the maximum period of observation The percentage of positive inoculations was roughly proportionate to the number of gonococci injected, being 93 per cent of eyes infected with approximately 20,000,000 gonococci and falling to 45 per cent in eyes injected with approximately 200 gonococci

Rabbits with persistent gonococcal infection developed positive complement fixing antibodies in their serum in a majority of instances, indicating a humoral immune response to this localized infection in the eye A few of the animals developed demonstrable agglutinins to gonococci, but never in significant titres

Although this experimental infection does not simulate the natural infection in man, it was used to test the effectiveness of gonococcidal agents in vivo These tests showed that 15 per cent sulfathiazole and/or 30 per cent calomel were highly effective when they were made up in watery or vanishing cream ointments, but not when they were made up in oily or greasy ointments

We are still left with the unsolved problem of producing gonococcal urethritis in some experimental animal

LONG TERM RESULTS IN NEUROSYPHILIS TREATED WITH PENICILLIN ALONE*

By

John H Stokes, Mortimer S Falk, and *George D Gammon*

with the collaboration of Herman Beerman, Norman R Ingraham, Jr, William O LaMotte, Paul Gyorgy, Elizabeth K Rose, and John W Lentz, and the technical assistance of Verna Mayer Stein Emily Stannard, Jane Barbara Taylor, and Mildred F Wills†

The Penicillin Syphilis panel at the University of Pennsylvania has rendered annual reports of the effect of penicillin alone on neurosyphilis since 1943 (1) (2) (3) Observation of some of the total of 537 cases treated has now reached the fourth year and longer, and the serial examinations of spinal fluids and parallel clinical data give an impressive account of what the antibiotic actually accomplished not only of itself, but in comparison with the methods of therapy heretofore available Particularly is this so when the laboratory has maintained uniform practices throughout a period of many years, and the clinical staff has been expert and stable The case material is now sufficiently large so that the graphs which follow will deal only with material observed two years or more

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This investigation also was assisted by contributions from the Gar Wood Fund and the Kirby McCarthy Fund

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Fig 1

Progress of the Original 39 Cases of Neurosyphilis toward Spinal Fluid Normality Regardless of Type of Neurosyphilis Dose or Number of Courses of Penicillin

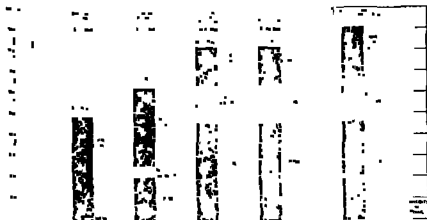


Figure 1 brings up to date (March 1948) the spinal fluid record of our 39 "Original Inhabitants," the first cases treated with penicillin in 1943, of whom 32 have been followed for 3 to 4 years Recall the terminology we use "spinal fluid normal" and "spinal fluid near-normal" (cell count 5 to 10, total protein 30 40, colloidal test 4 or more 1's, Kolmer Wassermann 0000), and "marked improvement," which means improvement in all four items of the fluid examination

From this graph it is clear that spinal fluid normality and near normality has been maintained at a level of 47 and 19 per cent respectively, or 66 per cent combined Marked improvement with the passing of another year has increased from 18 per cent to 25 per cent

Figures 2, 3 and 4 are extensions through another year of graphs published last year In Figure 2 it will be apparent how few patients fail to achieve spinal fluid normality or near-normality in asymptomatic neurosyphilis Occasional failure and relapse is, however, an undoubted fact, while on the other hand, initial failure to respond may eventuate in a good result

Figure 3, combining clinical paresis and tabo paresis, well illustrates the poorer prognosis of the later and more serious

FIG 2

BEHAVIOR OF THE SPINAL FLUID IN ASYMPTOMATIC NEUROSYPHILIS (TYPE III FLUID FORMULAS) FOLLOWING 1 TO 3 COURSES OF PENICILLIN REGARDLESS OF DOSE OBSERVED 2 YRS OR MORE

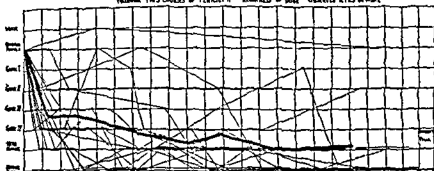
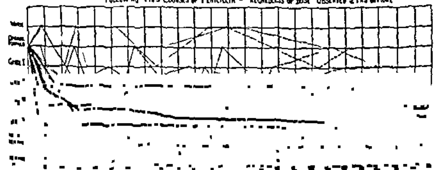


FIG 3

BEHAVIOR OF THE SPINAL FLUID IN CLINICAL PARESIS AND TABOPARESIS WITH TYPE III FLUID FORMULA FOLLOWING 1 TO 3 COURSES OF PENICILLIN — REGARDLESS OF DOSE OBSERVED 2 YRS OR MORE



manifestations of clinical neurosyphilis Nonetheless the prolongation of the curve of best fit, more dependable in this material observed over a longer period, indicates a steady betterment of the outlook with passage of time, and a low incidence of relapse in the spinal fluid

Figure 4, presenting tabes dorsalis with Type III (resistant) spinal fluids, shows a distinctive difference in behavior between paresis and taboparesis, and uncomplicated tabes The rapid and complete response of abnormal fluids in tabes compares with that in asymptomatic neurosyphilis and suggests a biological difference in the paretic as compared with the tabetic type of process This is of course in line with the spontaneous trend toward fluid normality with clinical progression familiar as part of the tabetic picture

Figure 5 tabulates in case numbers and (with apologies) in percentages the normal and near-normal spinal fluids achieved in the various categories of neurosyphilis among patients ob-

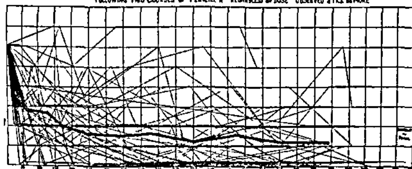


Figure 5

NEUROSYPHILIS OBSERVED 2 YEARS OR MORE

DIAGNOSIS	TOTAL CASES	NO CASES NEAR NORMAL C S F	NO. CASES NORMAL C S F	TOTAL CASES NORMAL AND NEAR NORMAL C S F
PARESIS	11	3 - 27%	2 - 19%	5 - 46%
TABO PARESIS	20	2 - 10%	10 - 50%	12 - 60%
TABES DORSALIS	55	15 - 27%	29 - 53%	44 - 80%
MENINGOVASCULAR	33	5 - 15%	15 - 45%	20 - 60%
ASYMPTOMATIC	23	7 - 30%	10 - 44%	17 - 74%

rved for more than two years The highest proportions (80 r cent and 74 per cent) are attained in tabes and in asymptoatic neurosyphilis respectively The showing in paresis and boparesis is definitely higher than that reported for 1946, and 60 per cent for 1947 as compared with 35 and 39 per cent r 1946 This gain emphasizes that time as in malarial therapy, part of the mechanism of recovery The showing of penillin within the limitations of the previous tabular computaons, has become even more markedly superior to that of malaria far as the spinal fluid is concerned Tabes and asymptomatic eurosyphilis have registered still more progress toward normaly with another year, from 55 to 80 per cent in tabes and from 1 to 74 per cent in asymptomatic neurosyphilis Meningovascuar neurosyphilis has progressed toward fluid normality least of ll (55 to 60 per cent)

PRESENTATION OF CASE PROTOCOLS *We have selected the protools for this presentat on of type forms of response to penicillin*

with no thought for the statistical comparison of this with that, but solely with intent to illustrate graphically the sequence of events in the individual case The periods of observation are in the main long, and make it possible to observe the factors of course repetition, time and comparative symptomatic and serologic responses Examples of failure are offered with those of success Comparisons are possible between low dosage of the earlier penicillin and the higher later dosages of the refined product One can derive an impression of how much how little penicillin is really able to accomplish It is possible to compare in individual cases, the effect of penicillin as compared with that of the routine intensive arsenic heavy metal therapy of the past, with intraspinal therapy, with tryparsamide, and with fever therapy, where fever has failed The Dattner Thomas inactive type fluid sometimes unaffected by penicillin, sometimes responsive to it, is represented Finally, two records representing 22 and 26 years of treatment and observation by one of us (JHS), close the series The penicillin effects observed are those of penicillin alone, administered as the water soluble sodium or calcium salt in a round the clock hospitalization system

The first column (left) of each protocol gives dates, the second days of observation since the first penicillin injection The Kline titer or regain in the blood is given in units The spinal fluid (next four columns) includes cells a quantitative Kolmer Wassermann, total protein estimated by the Kingsbury Clark method which tends to read low in comparison with the electrophotometric method and a colloidal mastic test The earlier protein reports are in terms of Noguchi and Pandy technics and the colloidal benzoin test appears in early Mayo Clinic findings Normal cell counts are 4 or less normal protein 10 to 30 mgm per cent negative Kolmer is 0000 and normal colloidal mastic is 0000000000 to 1110000000

The penicillin dosage line gives the total dose, the hourly interval and the inclusive dates of the treatment

Figures 6 and 7 represent the response of asymptomatic neurosyphilis to penicillin in virtually previously untreated patients with Type III fluids (resistant) It is assumed that the third course was necessary in Figure 7, though time might have produced a normal fluid after the second course

Note that effects are secured in days that formerly required weeks and even months especially on the cell count, and note

FIG 6

RESPONSE OF ASYMPTOMATIC NEUROSYPHILIS

#3 L.S., W.F.41, 11/23/43
Asymptomatic neurosyphilis
Previous treatment: none

No clinical evidence of CNS syphilis.
CSF positive, October 1943.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
(1)	1,200,000 units penicillin (every 3 hours) 11/27/ to 12/5/43						
11/27/43	1	64	103	4444	4+	2444411000	
12/14/43	17	8	29	1244	2+	2221100000	
1/12/44	46	32	11	0012	1+	2211000000	
2/ 9/44	74	32	6	0112	30	1111000000	
3/ 8/44	102	32	6	0112	40	2211000000	
4/ 5/44	129	8	4	0011	30	1111000000	
5/ 5/44	159	32	8	0122	30	2211100000	
5/24/44	178	16	6	0012	30	2221100000	
(2)	2,400,000 (every 3 hours) 5/28/ to 6/2/44						
5/28/44	182						Urticarial reaction
6/ 2/44	186	4	7	0000	20	1111000000	
10/ 4/44	310	32	3	0000	30	1110000000	
12/13/44	380	32	3	0000	20	1111000000	
3/ 7/45	464	8	3	0000	40	1110000000	
6/27/45	576	32	6	0000	20	0000000000	
1/ 9/46	779	64	2	0000	20	0000000000	
6/19/46	935	32	8	0000	20	0000000000	
1/ 5/47	1200	2120	3	0000	10	0000000000	Asymptomatic

FIG 7

#46 J.W., B.M.26, 2/9/44
Asymptomatic neurosyphilis
Previous treatment: 8 arsphenamine, 8 bismuth

No clinical evidence of CNS syphilis. CSF positive (Draft Board). Duration of disease 27 years

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
1/26/44	Pre Pen.		68	4444	30	4431100000	

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
(1)	1,200,000 units penicillin (every 3 hours) 2/9/ to 2/13/44						
2/ 9/44	1	2					
2/14/44	5		42	4444	40	2211000000	
3/16/44	29	4	4	0122	30	2332210000	
5/ 9/44	71	32	RBC	4444	30	4432110000	
6/21/44	127	2120	18	4444	40	3554221000	
7/18/44	154	Neg.	7	4444	20	2333211000	Physical exam. negative
(2)	2,400,000 (every 3 hours) 8/7/ to 8/15/44						
8/ 7/44	174	16					
11/ 8/44	267	4	8	0011	30	1111100000	
1/ 3/45	323	4	5	0000	20	1111000000	
4/ 4/45	416	8	5	0112	20	2221100000	
(3)	10,000,000 (every 3 hours) 5/18/ to 6/18/45						
5/18/45	459	16					Physical exam negative
5/22/45	463	16	4	0012	30	2221100000	
8/ 1/45	535	120	2	0000	10	1110000000	
10/24/45	620	64	2	0000	20	1111000000	
1/23/46	713	16	2	0000	20	1110000000	
5/ 1/46	812	64	2	0000	20	1110000000	
1/23/47	1079	4					
5/14/47	1192	2	0	0000	20	1110000000	Asymptomatic

too in these and other protocols, the non-responsiveness of the STS.

Figures 8 and 9 present a success and a failure (?) in congenital neurosyphilis Figure 8 responded to penicillin after the failure of arsenicals and fever Figure 9 probably represents an arrest at a low level of deterioration of an almost untreated juvenile paresis The fever is by typhoid inoculation after penicillin failed to better the condition of the patient

Figure 10 presents the Dattner-Thomas type of spinal fluid consistent from start to finish over 1470 days and almost the homologue of treatment fastness in the blood The cell count has however reached 5 and total protein 40 mgm on 2 occasions in 1944-48 The total amount of penicillin is 10,000,000 units in 3 courses There has been no symptomatic progression Note the large amount of pre penicillin therapy including 15 plus tryparsamide injections, and hot box fever

Fig 8

A PENICILLIN AND FEVER FAILURE, CLINICAL AND SEROLOGIC

(CONGENITAL PARESIS)

#7 W L , B,F.14, 11/27/43
Late congenital CNS syphilis, paresis
Previous treatment: 2 Arsphenamine and 15 bismuth injections

Personality changes with impaired intelligence and memory. Irritability, emotional instability, uncertain gait (8 months duration). Pupils irregular-fixed to light and accommodation Hutchinsonian teeth Slurred speech EEG: suggestive of diffuse disturbance of greater part of cortex.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS	PROT	MASTIC	CLINICAL
9/ 8/43	Pre Pen.		12	4444	4+	5555431100	
(1)	1,200,000 units penicillin (every 3 hours) 11/27/ to 12/5/43						
11/27/43	1	64	42	4444	4+	5555311000	
2/ 2/44	64	64	4	1244	2+	4421000000	
(2)	3,600,000 (every 3 hours) 2/17/ to 2/25/44						
2/17/44	77	64					
2/25/44	85	32	5	4444	20	5552100000	
4/ 5/44	127	22	3	2444	40	5555310000	No improvement
5/17/44	168	32	1	4444	40	5555221100	
10/21/44							Deteriorated - worse
1/16/45	408	64					
(3)	4,000,000 (every 3 hours) 1/28/ to 2/15/45						
2/ 15/45	433	128					
Given typhoid, 45 hours at temperature over 103°							
5/19/45	470		4	4444	30	4443110000	
7/25/45	598	64	6	0112	10	2221100000	Improved- EEG almost normal Very retarded mentally
10/25/45	696	256	4	4444	20	5553310000	Seems stationary
6/ 1/46	891	256	0	0112	10	2221100000	No great change
11/20/46	1088	256	5	4444	10	4443210000	
9/10/47	1384	256	0	0112	10	2221100000	Mentally W 1

FIG 9

#11, R.C., W.M.18, 12/9/43
Late congenital CNS syphilis, primary optic atrophy
Previous treatment: much arsenical and fever

Hutchinsonian teeth, slight frontal bosses, slight arching of palate; Higoumenakis positive, anisicoria, OD sluggish to light, OS fixed. Ptosis of left eyelid. Left N.VII cranial nerve--muscle (facial) weakness. Reflexes sluggish. Slight contraction of visual fields.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
11/16/43	Pre Pen.		32	1244	4+	3332210000	
(1)	1,200,000 units penicillin (every 3 hours) 12/9/ to 12/18/43						
12/ 9/43	1	32					
12/22/43	13		16	0122	1+	2111000000	
1/14/44	40	4	6	0011	±	1111000000	Achilles diminished, cranial nerve OK (Gammon)
4/19/44	140	2±	1	0000	30	1110000000	
(2)	2,400,000 (every 3 hours) 7/28/ to 8/13/44						
7/28/44	240						
12/14/44	370	Neg.	1	0000	10	1110000000	Early P.O.A., fields slightly contracted
7/ 5/45	573	Neg.	3	0000	20	0000000000	No change in eye examination
1/30/46	782	1±	0	0000	20	0000000000	
(3)	10,080,000 (every 3 hours) 3/11 to 3/25/46						Progression of P.O A.
3/11/46	825	Neg.					
3/26/46	839	Neg.	2	0000	20	0000000000	No change in eye examination
6/ 5/47	1277	Neg.	0	0000	20	0000000000	

Figure 11 portrays the temporary response of the spinal fluid in meningo-vascular neurosyphilis with apparent relapse after 14 4 million units of penicillin in 4 courses Note the failure of 12 arsenical, 86 bismuth and 91 tryparsamide injections to bring the fluid to normal

Figure 12 affords an opportunity to compare the efficiency of routine arsenical-bismuth plus Swift Ellis intraspinal therapy with a minimal course (1 2 million units) of an early penicillin (1943) As much was accomplished by 1 2 million units of this penicillin as by 14 Swift-Ellis treatments and 34 bismuth injections

Figure 13 presents a symptomatic result in tabetic pain Note that the negative spinal fluid is not consistent In the emphasis often placed *on its remarkable effect on the fluid*, penicillin symptomatic response are sometimes under-estimated Patient has been free of pain for 40 months

Figure 14 presents Herxheimer effects from initial maximum dosage of penicillin and a striking and lasting (2 year) serologic and clinical response in general paresis, following 2 courses of penicillin, totalling 3 6 million units Total observation, over 4 years

FIG 10

DATTNER-THOMAS INACTIVE TYPE FROM START TO FINISH;

THREE AND ONE-HALF YEARS, 9 MILLION UNITS

#22, M T., B.F.47, 1/4/44
Meningovascular syphilis, primary optic atrophy (?)
Previous treatment: 53 arsphenamine 15+ tryparsamide, 104 bismuth, and fever (hotbox).

History of positive CSF 1940
Argyle-Robertson pupils
Lyperreflexia

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
(1)	1,200,000 units penicillin (every 3 hours) 1/5/ to 1/13/44						
							[illegible] of P.O A (Steele says no.)
(3)	6,400,000 (every 3 hours) 11/17 to 11/27/45						
11/ 7/45	654	Neg.					
11/14/45	661	Neg.	1	4444	30	4554421000	
2/20/46	776	Neg.	5	4444	30	4555311000	
7/23/46	930	Neg.	1	4444	40	4421100000	No evidence of P O A (LaMotte)
1/ 8/47	1101	Neg.	0	4444	30	5555553100	
6/18/47	1262	Neg	2	4444	30	4555521000	Some vertigo; pains in legs? Given aspirin; Discs, vision and field examinations normal
1/14/49	1470	Neg	2	4444	20	5554321000	Eye and Neurol. no change.

Figure 15 presents paresis with marked clinical improvement without corresponding spinal fluid response, the patient remaining in an approximation to the Dattner-Thomas inactive status Unfortunately his wife did not agree with the neurologists on his mental status Total penicillin 16 0 million units in 3 courses

Figure 16 presents a clinical tabo-paresis with some of the serologic behavior of a resistant meningovascular type Total 10 million units in 3 courses

Figure 17 presents a taboparesis showing lasting serologic improvement under penicillin, with temporary marked clinical improvement followed by clinical relapse Fever (typhoid inoculation) and electro-shock used to improve the clinical results with some response less depression but still psychotic Penicillin 15 million units, fever 52 hours over 103 degrees

FIG 11

A COMPARISON OF PENICILLIN AND TRYPARSAMIDE IN A PATIENT WITH MENINGOVASCULAR SYPHILIS

#30 A E., B.F.58 1/13/44
Meningovascular syphilis
Previous treatment: 12 arsphenamine, 86 bismuth, 91 tryparsamide

Mentality dull; Argyle-Robertson pupils, diminished deep reflexes. Poor memory and slurred speech.

DATE	DAYS POST PEN	KLINE	CELLS	WASS.	PROT	MASTIC	CLINICAL
1/11/44	Pre Pen		6	4444	3+	2355331000	
[illegible]							
(2)	2,400,000(every 3 hours)8/2/ to 8/17/44						
8/ 2/44	201						No change
9/13/44	243	Neg	2	2344	30	3444220000	
10/ 3/44	262	21±0	2	1244	30	3444210000	
2/13/45	395	Neg.	3	1244	20	2444111000	No change
(3)	1,200,000(every 3 hours)4/10/ to 4/18/45						
4/10/45	420	Neg.					
4/18/45	428	Neg.	6	2344	40	2443311100	
5/22/45	462	Neg.	0	1244	30	4444211000	
10/31/45	656	Neg.	2	1244	50	3444211000	No change
1/ 2/46	719	Neg.	RBC	4444	40	3444321100	
4/17/46	826	Neg.	2	1244	30	2233221000	
7/10/46	910	Neg.	2	1244	30	1122211000	
11/27/46	1051	Neg.	4	4444	20	2444421000	No change
(4)	9,600,000 (G)(every 2 hours)1/10/ to 1/20/47						
1/10/47	1096	Neg					BP 245/126--essential hypertension
1/15/47	1101		1	0000	30	1111000000	No change--neuro.
1/20/47	1106	Neg	2	0000	30	1111000000	No change
9/17/47	1344	Neg.	3	1244	30	2444210000	No change
3/10/48	1517	Neg.	2	4444	40	2344431000	No change

Figure 18 presents a demonstration reinforced by graphic evidence, of the clinical and serologic effect of penicillin in a succession of courses on a patient with tabo paresis and primary optic atrophy Total penicillin 16 4 million units in 4 courses Note the changes in the sector defects in the visual fields suggesting localized or "island" relapse (comparable to neuro recurrence?) and their apparent response to more penicillin The slight fluctuations in "normal" and "near normal" fluid formulas are apparent, even the latest colloidal test being "near" by our nomenclature (four one's in the colloidal test) We interpret such slight variations as of questionable pathologic significance, but requiring continued observation The euphoria and irritability have been improved by treatment

Figure 19 presents one of our best results in the penicillin

FIG 12

#12 J L. W M 51 12/14/43
Tabes dorsalis with primary optic atrophy
Previous treatment: 14 neoarsphenamine 70 bismuth and Swift Ellis

Diminution of vision (4 years) progressive; pupils irregular, primary optic atrophy Tendon reflexes absent OD 6/9, OS light perception CSF positive 12/8/41

SWIFT-ELLIS

14 treatments + 54 bismuth; CSF examination with each Swift Ellis as follows:

DAYS	CELLS	PROT	WASS	MASTIC
1	52	4+	4444	3444210000
8	10	4+	4444	5442211000
15	9	2+	1244	3332210000
27	6	1+	0124	2220000000
59	16	3+	1244	4442111000
75	12	3+	1124	4442210000
131	4	Neg	0000	1110000000
144	3	Neg	0000	0000000000
	2	Neg	0000	1110000000
202	2	35	Pos	1112222000
216	2	Neg	0000	1110000000
230	2	←	0000	1111000000
250	3	←	0000	1111000000
264	4	←	0011	1111000000
395	2	Neg	0000	1110000000
SWIFT-ELLIS STOPPED FOLLOWED BY RELAPSE				
593	58	4+	4444	2444310000
625	61	2+	1244	2443100000

PENICILLIN

1 200 000 units begun 89 days after Swift Ellis relapse was recognized CSF findings follow:

DAYS	CELLS	PROT	WASS	MASTIC
1	74	4+	4444	3444210000
43	10	2+	0124	1221000000
85	7	30	0011	1111110000
127	9	30	1122	1222100000
183	10	20	0011	1111100000
287	5	30	0000	1111100000
413	0	20	0000	1110000000
501	2	30	0000	0000000000
701	RBC	30	0000	0000000000
1079	3	30	0000	0000000000
1359	2	30	0000	0000000000

AS MUCH WAS ACCOMPLISHED BY 1 200 000 UNITS OF PENICILLIN AS BY 14 SWIFT ELLIS AND 54 BISMUTH

A CASE DEMONSTRATION OF (1) SUPERIORITY OF 1 2 MILLION UNITS PENICILLIN (1943) OVER SWIFT ELLIS THERAPY; (2) RATE OF CSF RESPONSE (3) DURATION OF GOOD EFFECT CSF NORMAL 2 1/2 YEARS (4) P O A ARRESTED 3 1/2 YEARS (5) TOTAL OBSERVATION 6 YEARS 3 3/4 YEARS AFTER PENICILLIN

FIG 13

RELIEF OF LIGHTNING PAINS CSF NEGATIVE

#55 N P W M 38, 2/13/44
Tabes dorsalis, lightning pains
Previous treatment: 22 arsphenamine, 35 bismuth

Pains (several months) Tendon reflexes absent; pain sense lost in muscles, tendons; anisocoria; slight nystagmus

DATE	DAYS POST PEN	KLINE	CELLS	WASS	PROT	MASTIC	CLINICAL (PAIN)
(1)	1,200,000 (every 3 hours) 2/23/ to 3/2/44						
2/23/44	1	Neg	3	0000	30	1100000000	
3/ 2/44	8	2	3	0000	40	1111100000	
4/ 5/44	42	8	3	0112	40	1111110000	
5/10/44	77	Neg	RBC	1122	20	2443210000	
6/14/44	112	1 0	5	0212	30	2222100000	
7/12/44	140	1+0	2	0000	30	1111100000	
9/13/44	183	Neg	1	0112	20	2442100000	
(2)	1,200,000 (every 3 hours) 10/12/ to 10/16/44						
10/12/44	212	32	4	0011	20	1111000000	
10/30/44							
11/22/44	254	8	1	0112	30	2444311000	Pain gone Remarkable remission of pain
5/ 7/45	350	21+0	3	0000	40	1111000000	Marked improvement maintained
6/26/45	471	1+0	3	0000	30	1110000000	
11/ 7/45	622	1 0	1	0000	40	1110000000	No recurrence
3/27/46	767	2	2	0000	40	1111000000	
1/ 7/47	1050	2	3	0112	40	2221100000	No tabetic pain
7/16/47	1239	2+0	2	0000	50	1111000000	No pain
1/14/48	1415	32	0	0000	40	1110000000	No pain

FIG 14

CLINICAL AND SEROLOGIC RESPONSE OF PARESIS

#34 C.P., W F 37, 1/19/44
Paresis; previous treatment (?) 6 mos. 10 years ago.
Duration of disease (?)

Accident precipitated present condition (3 months duration). Memory loss, speech difficulties; personality change; marked tremors, hyperactive tendon reflexes; EEG, cortex disturbance; Eye, normal fundi, fields not done.

DATE	DAYS POST PEN	KLINE	CELLS	WASS	PROT	MASTIC	CLINICAL
1/ 7/44	Pre Pen.		90	4444	74	2555552210	
(1)	1,200,000 units penicillin (every 3 hours) 1/19 to 1/29/44						
1/ 19/44	1	64					Reaction? Convulsion on second day.
1/ 31/44	13	64	34	4444	4+	4555531100	
3/ 30/44	71	64	6	4444	50	2443310000	
5/ 10/44	112	8	9	4444	40	4555211000	
6/ 28/44	161	21+0	0	0112	30	2221100000	
10/11/44	266	64	4	1233	40	4442100000	Much improved
(2)	2,400,000 (every 3 hours) 1/22 to 2/1/45						
1/ 22/45	370	64					
1/ 31/45	379	32	6	4444	40	2444431100	
5/ 9/45	477	1+0	1	0000	40	1110000000	
9/ 18/45	610	?	6	0000	40	1110000000	Clinical remission sustained.
1/ 23/46	734	2	1	1244	20	5542110000	Mentally practically normal.
5/ 8/46	840	16	2	0000	20	1110000000	
6/ 19/46	882	64	2	0000	20	1110000000	
11/13/46	1089	16	1	0000	20	1110000000	Irritable (domestic difficulties?)
9/ 3/47	1322	21+0	2	0000	20	0000000000	Mentally better; present disturbance not due to syphilis. (Gammon)
3/10/48	1511	21+0	0	0000	20	1110000000	Improvement maintained

therapy of general paresis, a remission with restoration to working capacity and reduction of a typical Type III spinal fluid to normal Spinal fluid normality has been maintained 13 months and clinical improvement 4 years

TWO LIFE HISTORIES OF NEUROSYPHILIS To understand the place and worth of treatment in a chronic disease such as syphilis of the nervous system, it is desirable as rapidly as pos sible to collect and publish a series of background protocols, of which Figures 20, 21, 22, and 23 24 25 are representative They portray respectively over a period of 22 years observation of a 36 year old infection, and 26 years observation of a 40 year-old infection, the course of neurosyphilis in 3 phases of treatment

The first mentioned patient has been treated with the arsenicals including tryparsamide and heavy metal, with therapeutic malaria, and finally as his spinal fluid again became definitely abnormal with penicillin The ultimate outcome under penicillin cannot yet be determined The "slipping" of an apparently

FIG 15

CLINICAL REMISSION (?) WITHOUT SEROLOGIC "CURE"

DATTNER-THOMAS "INACTIVE STATUS"

#74 H G., W M.43, 3/28/44
Paresis, previous treatment; bismuth only
Duration of disease: less than 18 years

Mental deterioration (5 years), progressively worse; delusions of grandeur, emotional lability; euphoria; impotence, dysarthria; shuffling gait; pupils sluggish; hyperesthesia of soles.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT	MASTIC	CLINICAL
	Pre Pen.		62	4444	100	5555555431	
(1)	4 million unit's penicillin (every 3 hours) 3/28 to 4/13/44						
3/28/44	1	64					
4/23/44	27	32	4	4444	50	5555543210	
5/24/44	56	64	3	4444	50	5555544221	
6/26/44	61	32	5	4444	40	4555554321	
11/2/44	217	32	?	4444	20	4555542111	
3/21/45	356	4	4	4444	30	5555542110	Definite improvement
(2)	2,400,000 (every 3 hours) 4/3 to 4/11/45						
4/ 3/45	369	4					
5/16/45	412	Neg.	5	4444	40	4555542110	
7/31/45	487	Neg.	1	4444	40	4555541100	
10/31/45	582	64	3	4444	20	4555332100	Symptomatic improvement
1/ 2/46	645	8	6	4444	40	5555431100	
4/17/46	750	32	2	4444	30	3555*22100	
10/23/46	947	16	3	4444	40	3554211000	Improvement maintained
(3)	9,600,000 (every ? hours) 11/13/ to 11/23/46						
11/13/46	962	4					Euphoric but not obnoxious
11/20/46	976		1	4444	30	4555431000	
5/20/47	1151						Improvement: General B-4, euphoria B-1, mental B-3
9/10/47	1258	32	0	4444	40	2455441000	(1) Greatly improved; no real indication he ever had paresis now (Churchill). (2) Wife thinks no better than pre-Pen.
12/31/47	1374	4	1	1244	30	2344420000	Improvement maintained (Churchill)

good malarial result began in the 14th year after the seige. It was serologically repeatedly confirmed and within 2 years was accompanied by slight symptomatic signs which improved with the second course of penicillin The fluid still remains slightly but not definitely abnormal

The patient represented by Figures 23, 24, 25 is portrayed in 3 phases of treatment, the first for intractable lightning pains and a type III spinal fluid, treatment covering 9 months with 37 arsenical injections, 62 injections of mercury succinimide, mercurial inunctions, sodium iodide intravenously, and 8 Swift-Ellis Ogilvie intraspinal treatments The therapeutic effect was negligible The second phase consisted of tryparsamide

FIG 16

AN EXAMPLE OF UNRESPONSIVE BLOOD, CSF RESISTANCE AND CLINICAL PROGRESSION, IN SPITE OF PENICILLIN

#43 A.K., W.M.(age?) 2/7/44
Taboparesis
Previous treatment: 30 arsphenamine, 50 bismuth.

Routine STS positive 1942. Positive CSF 1/3/44. Anisiscoria, coarse tremor of tongue. Mental: station, gait and reflexes O.K.

DATE	DAYS POST PEN	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
1/ 3/44	Pre Pen.	-	1	0122	2+	1233310000	
(1)	1,200,000 units penicillin (every 3 hours) 2/7/ to 2/11/44						
2/ 7/44	1	8					
2/23/44	18	1±0	5	4444	20	2444111000	
3/29/44	45	32	3	0122	20	1221000000	
5/ 3/44	80	2	RBC	0124	20	2443210000	Tremor of tongue and anisiscoria; otherwise negative.
10/ 3/44	233	21±0	4	0012	20	2221100000	Slight ataxia; impression: no change(?)
1/30/45	352	16	0	0112	20	2221100000	
(2)	2,400,000 (every 3 hours) 5/4/ to 5/11/45						
5/ 4/45	447	21±0					Somewhat unstable; has roaring in head.
5/ 8/45	451		3	0012	20	2221100000	
6/12/45	486	32	3	0000	10	0000000000	
11/ 5/45	636	Neg.	3	0000	20	1110000000	Continues to have roaring in head. Romberg sl. positive.
2/27/46	750	21±0	3	0000	20	1110000000	Has had some hallucinations. Impression: paresis worse.
(3)	6,400,000 (every 3 hours) 3/29/ to 4/8/46						
3/29/46	781	32					
4/ 3/46	786		4	0000	20	1110000000	
1/22/47	1085	Neg.	3	0112	20	1222100000	Some pain in right calf; still has roaring in head.
9/ 3/47	1304	4	4	1244	20	2344210000	No change (?)
2/25/48	1479	Neg.	2	0000	20	0000000000	No clinical change.

and bismuth and covered a period of 21 years with a total of not less than 1300 grams of tryparsamide (418 injections) and 225 bismuth injections After the fifth year, in the second phase, the patient several times attained a negative spinal fluid, and reached the peak of his professional career about the 12th year in this treatment phase His spinal fluid then again showed recurring abnormality, personality change began to accompany *fatigue* and overwork, and by the final year of the tryparsamide phase in 1944 he had a classical active Type III fluid again, and though the pains had largely disappeared, it was clear that he was at times slipping mentally He was never given fever therapy because of a suspicion of coronary spasm

In 1944 the penicillin phase of his treatment was initiated with 2 4 million units At this time he showed definite depres-

FIG 17

SEROLOGIC WITHOUT CLINICAL IMPROVEMENT UNDER PENICILLIN

RESPONSE TO SHOCK THERAPY

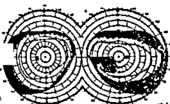
#62 R.X., W.F.47, 3/3/44
Taboparesis
Previous treatment: none

Past year: tremors, increasing nervousness, emotional lability, disoriented, slurred speech, paresis left arm, ptosis left eyelid. Pupils fixed to light, absent achilles and patellar reflexes; absent vibratory sense. EEG normal.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
2/23/44	Pre Pen.	Neg.	10	4444	40	4555543100	
(1)	3,000,000 units penicillin (every three hours) 3/3/ to 3/17/44						
3/ 3/44	1	Neg.					
4/19/44	46	2110	5	4444	20	5554311000	Improved in all aspects
6/ 7/44	90	Neg	7	4444	20	2554211000	Speech OK; does housework
10/13/44	215	Neg.	RBC	4444	20	3444221000	Still greatly improved; practically normal.
2/28/45	356	Neg.	4	1233	30	2444431100	Improvement maintained
(2)	2,400,000 (every 3 hours) 4/20/ to 4/28/45						
4/20/45	407	Neg.	4	4444	20	4442100000	
6/12/45	465	Neg.	0	0000	10	1110000000	
11/ 7/45	615	Neg.	2	0000	20	0000000000	Mentally clear; generally much improved; apparent cure (Todd)
2/27/46	726	Neg.	RBC	0000	10	0000000000	Talkative, irritable,
10/23/46							slurred speech (Culberson)
1/ 7/47	1049	Neg	2	0112	40	2221100000	No obvious mental deficiency.
(3)	9,600,000 (every 2 hours) 4/14/ to 4/25/47						Worse--delusions, hallucinations
4/14/47	1147	Neg	ND	0000	10	1110000000	Weight loss; impaired memory.
4/24/47	1157	Neg.	ND	0000	10	0000000000	No improvement
GIVEN 52 hours (typhoid vaccine) at temperature over 103°							
7/16/47	1232						
9/10/47							Discharged to institution for shock therapy
12/ /47							Dr. Gammon reports patient improved by shock therapy. Patient still psychotic but not depressed, better than pre-shock.

sion, had dizzy spells, his speech was slurring, the nasolabial folds flattened By the 94th day post penicillin the speech was improved, by the 275th day he spoke normally and was again incisive and alert At the 423rd day however, pains and over work had started him to slipping a little again, but he achieved the first normal spinal fluid in 17 years at the 556th day His 3rd penicillin course kept his fluid normal to the last examination on the 965th day, but he declined mentally to the point where he resigned his position, though still writing a good letter He died quite suddenly with symptoms of cerebral hemorrhage at the 1279th day post penicillin, at the age of 62

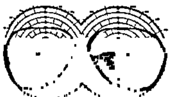
FIG. 18



TABOPARESIS WITH PRIMARY OPTIC ATROPHY

#3 E.P. W M-36 11/17/43
Previous treatment: routine 18 months 1937-1938

Episode of blurred vision and diplopia in 1937, which cleared under routine therapy. Visual loss recurred 9 months before admission. Pupils irregular and sluggish. Visual fields contracted; tendon reflexes absent; euphoria, irritability.



← PRE-PENICILLIN FIELDS

DATE	DAYS POST PEN	KLINE	CELLS	WASS.	PROT.	MASTIC
11/ 3/43	Pre Pen		72	4444	4+	4432210000
every 3 hours) 9/20 - 9/28/44						
04	16	11	0112	20	20	2211100000
32	2	5	0012	20	20	1111100000
64	120	5	0012	20	20	2211000000
50	2	2	0000	20	20	1110000000
every 3 hours) 10/8 - 10/16/44						
13	Neg.	4	0112	10	10	3332100000
		3	0012	10	10	2221100000



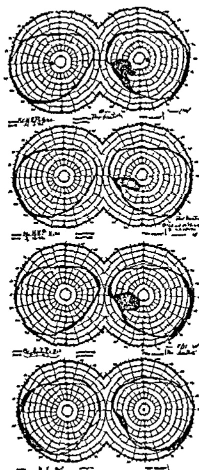


If the phases of this patient's treatment could have been shuffled, and his penicillin advanced to first place instead of last, before years of degenerative change and arteriosclerosis, toxic drugs and overwork had had their effect, it is not difficult to imagine the possible difference in result. Two years of penicillin in 3 courses totalling 11.4 million units accomplished as much or more than 23 years of routine arsenic-mercury therapy, intraspinal therapy and record totals of tryparsamide and bismuth.

SUMMARY

1. The fourth year of observation of the effect of penicillin alone on neurosyphilis sustains the record of the third year in our series, and shows a recognizable increase in the proportion of normal, near-normal and markedly improved spinal fluids obtained over previous reports.

FIG 18 (continued)



#5 E P, TABO PARESIS WITH P O A [illegible]

DATE	DAYS POST PEN	KLINE	CELLS	WASS	PROT	MASTIC
11/29/44	376	4	8	0112	30	[illegible]
8/28/45	469	±12	6	0112	90	[illegible]
3/15/46	646	Neg	3	0000	20	1100000000
5/ 7/47	1067	±12	6	0000	20	1122000000
9/17/47	1199		1	0000	10	1111000000

2 Forty six per cent of paretics, 60 per cent of tabo paretics, 80 per cent of tabetics with Type III spinal fluids have now achieved normal or near-normal spinal fluids

3 Sixty per cent of meningo vascular and 74 per cent of asymptomatic neurosyphilitics have achieved a similar status

4 Like the results presented in 1946, though to an even greater degree, these figures suggest the superiority in some types and at least the equality in others, of penicillin alone over malaria in neurosyphilis

5 A series of case protocols is presented displaying over periods of from 2 to 4 years and more various types of response success and failure in the therapy of neurosyphilis with penicillin alone

Fig 19

A GOOD RESULT, CLINICAL AND SEROLOGIC, IN PARESIS

#70 H.M., W.F.47 3/23/44
CNS syphilis, taboparesis
Previous treatment: none

Progressive gait changes (2 years); personality and mental changes (6 months), delusions, garrulous, *speech slurred. Unable to walk, gross tremors,* disoriented, achilles and patellar reflexes absent; anisocoria; EEG inconclusive. Total observation 1262 days.

DATE	DAYS POST PEN.	KLINE	CELLS	WASS.	PROT.	MASTIC	CLINICAL
3/17/44	Pre Pen.			4444	47	4432210000	Patient could not write or do housework. Had auditory hallucinations, personality changes, disorientation, tremor of tongue, hands and mouth, slurred speech. On second day of penicillin therapy, had Herxheimer reaction with right-sided convulsions becoming generalized. After 24 hours, penicillin was reinstituted at half-dose, to total 1.2 million units, without untoward effect. By 16th day, completely oriented, memory, speech, tremor improved; *EEG improved. In 4 months* patient tremor-free, speech and writing normal, well oriented, hallucination-free, and satisfactorily *performing housework,* including marketing with points and driving car.
		[illegible handwriting]					
(1)	4,000,000 units penicillin (every 3 hours) 3/23/ to 4/6/44						
3/23/44	1	64					
3/30/44	Hele F. Marroni						
4/23/44	25	64	6	4444	20	4443310000	
5/13/44	Helen L. marioni						
6/1/44	70	64	12	4444	20	5553211000	
7/11/44	110	64	1	0012	30	2221100000	
9/16/44	187	64	3	0112	20	2221000000	
10/6/44	Helen L Marioni						
4/17/45	392	128	1	0112	20	2221100000	
(2)	4,800,000 (every 3 hours) 9/17/ to 10/2/45						
12/18/46	1008	64	3	0012	20	2221100000	
2/6/47	1057	128	?	0000	10	0000000000	
(3)	2,500,000 (every 2 hours) 2/7/ to 2/17/47						THIS PATIENT SEEN 8 TIMES SINCE 10/6/44 AND TREATED WITH 13.4 MILLION UNITS ADDITIONAL PENICILLIN (3 COURSES). HAS A NORMAL FLUID (13 MONTHS) AND RATES A GRADE 3 TO 4 CLINICAL IMPROVEMENT, MAINTAINED.
9/3/47	1262	128	1	0000	30	1100000000	
Sept 3 1947 Dr Jammoni Helen L. Marioni Feeling very well							
3/3/48	1455	128	2	0000	20	0000000000	

6 Two "life histories" of neurosyphilis, covering 22 and 26 years of observation of 4 types of therapy including routine, arsenic heavy metal, malaria, tryparsamide and penicillin are presented Inferences can also be drawn from some of the protocols in the general series as to the relative effectiveness of penicillin as compared with older methods

7 Penicillin appears to be superior to other forms of treatment for all types of neurosyphilis, and certainly entitled on the score of safety, convenience and effectiveness, to first choice

Fig 20

LIFE HISTORIES OF NEUROSYPHILIS

(J H S Collection)

H W
Slide 1

(1) ARSEN L BI ... TR A D TRYPARSAMIDE PHASE

DATE	OBSERVATION	NUMBER OF TREATMENTS				STS	C S F				CLINICAL
		AS	TRYP	BIS	OTHER		CELL	WASS	PROT	COLLOIDAL	
11/9/26	Pre Rx					Pos	21	Pos	+	3221100000	Phy c l e an n ga [illegible]
12 8 27	1 yr	3+		3+	2	Pos	5	Pos		55433 0000	
10/25/28	2 yr		30			Pos	2	12444	3 Pandy	4555542100 JHH	Qu s ion Pe al y hang [illegible]
10/ /29	3 yr		50?			X ne Pos 1+	6 s me RBC	00	0	11100 0000	No s gn of de e o s ion
10/10/30	4 yr		40+			Neg	6	0012	+	3221000000	No change

Fig 21

L. E HISTO IES OF NEUROSYPHILIS (J H S Col tion)

H ?
S ?

2 THERAPEUTIC MALARIA PHASE

DATE	OBSERVATION	TREATMENTS			STS	C S F				CLINICAL
2 8/3	yr									CV ... In ed d y pul on ... murmur EFG normal, ... and increased
6/?/3					N ?					N ... og y ...
2/6/3	? yrs				N ?	5	0000	0	11 000000	N ... exam Neg ... CV no change
/3	? yr				N ?	5	0000	0	1110000?0	N progre on
?0/36	? yrs				Ne		?00	0	00?00	No pr gre on
/8/3	yrs				Neg					No p gre n
0/1 /38	? yrs				Kline	0	0000	0	1 0000000	N progr on
?? 39	13 yrs		20		N ?					Severe ... and ... no progre on
1? ?	yrs				Neg	2	0000	0	1 000000	N progr on
?/?0/	1? yrs				?o	0	0000	0	0000000	
? 30 3	yrs		20		N ?	1	0000	0	1 0000000	N p gre on
1?/1?/44	1? yrs				???					Que ... pup lary reaction ... CV No progre ion
11/1? ?	? yrs				Neg ?	?	0112	75	111 000000	Pup ... normal no progres ion
?/? ?	?0 yrs				Neg	0	0000	40	1 0000000	

? ... ... beginning ...

FIG. 22

LIFE HISTORIES OF NEUROSYPHILIS (J.H.S. Collection)

N.W.
Slide 3

(3) PENICILLIN PHASE

DATE	DAYS POST PEN.	PENICILLIN DOSAGE	STS	C.S.F.				CLINICAL AND SYMPTOMATIC
				CELL	MASS	PROT	COLLOIDAL TEST	
7/11/46	1	4.3 Mil. 7/11/ to 7/19/46	Dbt.					
7/19/46	8		Neg*					CV - no progression.
11/22/46	134		Neg.	2	0112	50	2221100000	
4/25/47	288		Dbt.	1	0112	50	1111000000	Some memory loss! Depression, mild. No definite progression of signs
9/26/47	442		Dbt.	1	0011	40	1111100000	
10/14/47	460	9.6 Mil. 10/14/ to 10/25/47	Dbt.					No progression.
10/25/47	471		Neg.					
2/13/49	581		Dbt.	2	0112	75	1111000000	No progression. Much improved mental tone.

* Boerner positive.

FIG 23

LIFE HISTORIES OF NEUROSYPHILIS

(J H S. Collection)

N Y.
Slide 1

CENTRAL NERVOUS SYSTEM SYPHILIS. LIGHTNING PAINS, PARESTHESIA AGE 35 6/25/21. DURATION OF INFECTION 14 YEARS WHEN OUR RECORD OF TREATMENT BEGINS TOTAL DURATION 40 YEARS DURATION OF TREATMENT 26 YEARS PREVIOUS TREATMENT: 2 INTRASPINAL; 30 MERCURY INTRAMUSCULARLY AND MERCURY INUNCTION

DATE									
6/25/21					Neg	12	Pos	Zone 1 (Gold)	
7/ 6/21	2	5	1	1		14	Pos	5555431100	Intractable lightning pains
7/28/21	3	10		1	Neg	14	Pos	Not done	
8/10/21	2	4		1		2	Pos	3331112220	
8/24/21	1					10	Pos	0455555331	Leg pains somewhat decreased.
	Hg Ungt - Rest								
2/ 9/22	1				Neg	6	Pos	5555552100	
2/18/22	3	5	3	1		38	Pos.	55[illegible]4[illegible]21[illegible]00	
3/ 4/22	4	6	6	1	Neg	3	Pos	1[illegible]55421000	No personality changes
3/18/22	4	7	5	1		3	Pos.	0233310000	
4/12/22	2	9	8						Worst problem, lightning pains

Fig. 24

[illegible] (J.H.S. Collection)

[illegible]

(B) [illegible]

DATE	[illegible]	[illegible]				STS	C.S.F.				CLINICAL AND [illegible]
		As	[illegible]	Tryp	Bis		Cell	Wass	Prot	Colloidal Test	
4/17/23						Pos	47	Pos		003300000000000	
6/20/23		0	0	10	0	Pos	6	Pos		Not reported	
[illegible]/23		0	0	1	0		[illegible]	Pos		[illegible]	
10/27/23		0	[illegible]	[illegible]	0	Neg	5	Neg		[illegible]	
[illegible]/23		0	0	1	0	Neg	[illegible]	Neg		[illegible]	
[illegible]											[illegible]
1/24/44	20 yrs	0	0	[illegible]	0	Pos	18	4444	40	[illegible]	Occasional leg pains. Mental set-up slips at times.
	TOTAL	[illegible]	14	[illegible]	[illegible]						

Fig 25

LIFE HISTORIES OF NEUROSYPHILIS (J.H.S. Collection)

N.V.
Slide 3

PENICILLIN PHASE

DATE	DAYS P ST PEN	PENICILLIN DOSAGE	STS	C.S.F.				CLINICAL AND SYMPTOMATIC
				CELL	WASS	PROT	COLLOIDAL TEST	
2/16/44	1	2.4 Mil. 2/16-2/26	32					Emotionally depressed, dizzy spells, speech slurring slightly, nasolabial folds flattened.
2/26/44	11		32	8	4444	40	2455310000	
3/17/44	30		32	6	1244	40	4433100000	
5/10/44	83		64	5	1124	30	3555221000	
6/20/44	94	3.0 Mil. 6/20-7/6	32	2	4444	30	4442210000	Speech improved, depressed but better.
7/5/44	109		32	6	4444	30	4444221000	
12/19/44	275		32	3	0000	20	1111100000	Marked subjective improvement Speech normal. Incisive, alert.
4/16/45	423		64	0	0112	30	4443220000	Slipping a little again. Pains.
9/7/45	566		64	4	0000	20	1110000000	
10/29/45	619	6.0 Mil. 10/29/ to 11/13/	64					
11/13/45	634		64	4	0000	30	1111100000	Urticaria
6/7/46	841		128	0	0000	40	1100000000	Writes a good letter.
10/11/46	965		128	1	0000	20	1111000000	Pains decreased 75% since penicillin. Holds his own. Resigned his job.
8/18/47	1279	Died. Symptoms of cerebral hemorrhage. Had failed professionally during terminal year.						

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THE TREATMENT OF NEUROSYPHILIS WITH A COMBINATION OF MALARIA AND PENICILLIN

By

*Robert R Kierland, Paul A O Leary,** and *Laurence J Underwood***

Penicillin is a valuable adjunct to the treatment of neurosyphilis However, there has been some difference of opinion regarding the value and desirability of its exclusive use in cases of neurosyphilis Because of the limited post treatment period of observation of patients with neurosyphilis treated with penicillin plus other methods, the reports as yet cannot be considered conclusive with regard to their relative values Malarial therapy of neurosyphilis, especially in late and more severe types has been demonstrated to be an important therapeutic measure[1]

In this report we wish to present the results of treatment with penicillin and malaria in a group of cases of late neurosyphilis These patients have been selected on the following basis 1 Only patients who received combined malarial and penicillin therapy have been included 2 Only patients who have been observed for a minimum of six months following combined treatment have been accepted The last examination of spinal fluid and the last clinical examination made prior to further therapy has been used as the basis for evaluation of the results If an individual received further treatment the observations previous to the time of further treatment were used for evaluation of the results of combined treatment

Many patients who had neurosyphilis have been treated by us with penicillin alone and with malaria alone and treated again later with penicillin alone So the results of treatment herein reported are the results obtained in a selected group of patients who had severe types of neurosyphilis and spinal fluid abnormalities and in whom the consultant felt that a combination of treatment with malaria and penicillin was necessary to produce the desired satisfactory result

Our series consists of 76 patients who were treated with penicillin and malarial therapy 59 of them were males (78 per cent)

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TABLE 1

Mater al

	Diagnos s						
	Paresis	Tabo-paresis	Tabes dorsal s	Pr mary optic atrophy*	Menin gov as cular neuro-syph l s	Asymp tomat ic neu rosyph ilis	Total
Pat ents total	19	16	13	7	8	13	76
Sex							
Males	16	12	10	7	5	9	59
Females	3	4	3	0	3	4	17
Age yr							
Less than 20	0	0	0	0	0	2	2
20–29	0	0	0	0	1	1	2
30–39	8	2	5	0	6	5	26
40–49	8	11	6	6	1	4	36
50–59	3	3	2	1	0	1	10

* Includes 1 patient with chor oret n tis

and 17 were females One patient was a Negro The following diagnostic categories were employed paresis, taboparesis, tabes dorsalis, primary optic atrophy, meningovascular and asymptomatic (Table 1) Of 7 patients who had primary optic atrophy, 3 had taboparesis and 4 had tabes dorsalis as well Two patients had congenital neurosyphilis One had active interstitial keratitis and asymptomatic neurosyphilis and the other had juvenile taboparesis One patient with asymptomatic neurosyphilis had a gumma of the frontal and right mastoid bones, which healed quickly after therapy One of the patients in the meningovascular group had cervical hypertrophic pachymeningitis There were no other concomitant syphilitic diagnoses Seventy two patients were 30 years of age or older, the majority (61 per cent) were 40 years of age or more The duration of syphilis was unknown in about 49 per cent, and in 41 per cent the disease was of more than ten years' duration (Table 2) Forty patients had had inadequate or no previous treatment and 30 had had twenty or more arsenical injections combined with heavy metal Only 4

TABLE 2

Duration of Syphilis

Duration	Paresis	Taboparesis	Tabes dorsalis	Primary optic atrophy	Meningovascular neurosyphilis	Asymptomatic neurosyphilis	Total
Unknown	7	11	7	3	4	5	37
5 yr or less	0	0	0	0	2	0	2
6–10 yr	0	0	0	0	1	5	6
11–15 yr	5	2	0	3	1	1	11
16–20 yr	2	0	3	0	0	2	7
More than 20 yr	5	3	3	1	0	0	12

TABLE 3

Previous Treatment

Previous Treatment	Diagnosis						Total
	Paresis	Taboparesis	Tabes dorsalis	Primary optic atrophy	Meningovascular neurosyphilis	Asymptomatic neurosyphilis	
None	6	9	5	1	3	3	27
Very little	3	1	2	3	3	1	13
20 treatments with arsenic and 20 with heavy metal or more	10	4	3	3	2	8	30
Penicillin or fever	0	2	3*	0	0	1*	6

* Fever treatment in 1 case

patients had previous penicillin Two patients had been given fever therapy previously, one by cabinet and the other with malaria (Table 3)

Penicillin was used in varying dosage and schemes The majority (75 per cent) of the patients were given a total of 4 000 000 to 6 000,000 units, usually 50,000 to 60,000 units were given intramuscularly every three hours for ten days Twenty seven patients were given crystalline penicillin G, the majority re-

ceiving 6 000,000 units Fourteen patients received less than 4 000,000 units of regular penicillin Five patients were given daily injections of penicillin in beeswax and oil, the total dosage varied from 3 600,000 to 6 000,000 units Concurrently, benign tertian malaria was used for all patients, 72 had satisfactory courses of fever The course of fever consisted of eight to ten paroxysms In 4 cases treatment with malaria was terminated prematurely One patient had pneumonia and another hypotension Two patients had spontaneous cessation of paroxysms, 1 of whom had had therapeutic malaria before

The length of observation after treatment ranged from six to forty four months Approximately 43 per cent were followed six months 40 per cent from six to eighteen months and 17 per cent eighteen months to three years or longer (table 4)

The importance of examination of spinal fluid in the proper evaluation of the condition of a patient with syphilis is recognized However, of those patients who had received previous treatment elsewhere 69 per cent had not had a previous examination of the spinal fluid The spinal fluids were grouped before combined treatment with malaria and penicillin according to the degree of abnormality in the following classification Group III represents the most active and abnormal ("paretic" formula) fluids group II the intermediate degree of abnormality, and group I the least abnormal fluids The grouping of the spinal fluids was

TABLE 4

Period of Observation After Treatment

Observation period, mo	Diagnosis						Total
	Paresis	Taboparesis	Tabes dorsalis	Primary optic atrophy	Meningovascular neurosyphilis	Asymptomatic neurosyphilis	
6	8	6	5	5	4	5	33
7 12	2	3	3	2	2	7	19
13 18	2	4	4	0	1	0	11
19 24	3	1	0	0	1	1	6
25 36	2	2	0	0	0	0	4
More than 36	2	0	1	0	0	0	3

derived from a combination of the results of the Kolmer complement fixation, colloidal gold and total protein tests and the cell counts A subcategory also was employed for the arrested type of spinal fluid pattern, that is, normal cell count of less than 5 cells per cubic millimeter, normal value for total protein of less than 40 mg per 100 cc with positive complement fixation The classification of the spinal fluid by groups according to the degree of abnormality may be seen in Table 5 Spinal fluids of 6 patients (8 per cent) were classed in group I, of 26 (34 per cent) in group II, of 40 (53 per cent) in group III, and of 4 (5 per cent) as the arrested type

RESULTS OF TREATMENT

SPINAL FLUID —The results are those obtained from a single course of treatment with malaria and penicillin Following this combined treatment with malaria and penicillin the condition of the spinal fluid was evaluated and compared with the condition after the initial examination The data given are a résume of the results of the last examinations of spinal fluid prior to any further treatment At this time group I fluids were present in 22 per cent of the cases, group II in 11 per cent, group III in 4 per cent and arrested type in 63 per cent All tests on the spinal fluid of 3 per cent of the patients gave negative results (Table 5) The grouping of spinal fluids before treatment revealed that the fluids of 13 per cent were in group I or in the arrested group while 85 per cent were in the same category after therapy

The most striking effect of successful treatment with penicillin alone or combined with malaria is the early return to normal of the elevated cell count in the spinal fluid This is followed closely or simultaneously by a return of the concentration of total protein to normal Later there is a gradual flattening of abnormal colloidal curves and the slowest response is the reduction of positivity of the complement fixation reaction

During recent years increasing emphasis has been placed on the cell counts and determinations of the total protein in the spinal fluid protein as a guide in the prognostication of arrest of the infection of the central nervous system and in the need for further therapy Dattner Thomas and Wexler[7] have emphasized the significance of the cell count and the determinations of total protein in observation of the treated patient They are of the opinion that these findings are of more value than a study of the clinical status in determining the arrest of the active disease process

TABLE 5

Spinal fluids classified in groups according to degree of abnormality

Group of spinal fluid	Diagnosis						Total
	Paresis	Taboparesis	Tabes dorsalis	Primary optic atrophy	*Meningovascular neuro-syphilis*	Asymptomatic neuro-syphilis	
Before treatment							
I	1(5%)	0	1(8%)	0	0	4(31%)	6(8%)
II	6(32%)	6(38%)	4(31%)	3(43%)	2(25%)	5(38%)	26(34%)
III	10(53%)	10(62%)	6(46%)	4(57%)	6(75%)	4(31%)	40(53%)
Arrested*	2(10%)	0	2(15%)	0	0	0	4(5%)
After treatment							
I	2(10%)	1(6%)	4(31%)	3(43%)	3(38%)	4(31%)	17(22%)
II	6(32%)	2(13%)	0	0	0	0	8(11%)
III	0	0	1(8%)	0	0	0	1(1%)
Arrested*	10(53%)	13(81%)	8(61%)	4(57%)	5(62%)	8(61%)	48(63%)
Normal	1(5%)	0	0	0	0	1(8%)	2(3%)

* Cell count less than 5 cells per cubic millimeter, total protein *normal or less than 40 mg* per 100 cc, positive complement fixation on Kolmer spinal fluid test variable result of colloidal test

It was found that 90 per cent of the patients in our series had more than 5 cells per cubic millimeter before treatment The cell count after treatment was more than 5 cells in 11 cases (14 per cent); in the majority of these cases between 5 and 10 cells per cubic millimeter were present In most patients the abnormal cell count was noted at examination six months after treatment in a few at twelve months There was no predominance of one type of neurosyphilis in patients with these abnormalities Three patients (4 per cent) had a relapse in cell count after an initial return to normal The relapses were in the borderline range (5 to 10 cells per cubic millimeter) and all within a year following treatment These occurred in patients who had paresis, taboparesis, or meningovascular neurosyphilis

Before treatment, the determination of total protein reveal values of more than 40 mg per 100 cc in 50 cases (66 per cent Determinations after treatment indicated that 19 patients (per cent) continued to have more than 40 mg of total protei but except for 2, all had decreased values from the levels befo treatment The majority of the abnormalities were noted at th six month examination, and the concentration of total prote was less than 75 mg per 100 cc at this period in all except cases There were abnormalities in each diagnostic catego with no definite predominance in any group

The quantitative Kolmer complement fixation test on t spinal fluid of only 3 patients (4 per cent) failed to show a d creasing titer in the posttreatment period Many times the d crease in titer was slight but definite Four patients (5 per cen had negative results from Kolmer tests from six to thirty mont after treatment One patient with tabes dorsalis and active pr gressing chorioretinitis had a more strongly positive Kolmer r action six months after treatment than before therapy, althou the results of the other spinal fluid tests were within norm limits

All patients with colloidal gold curves showing a reaction i the first zone or midzones showed a decrease in these valu after malaria and penicillin Of 57 patients with first zone (midzone colloidal gold curves before treatment, 53 per cent ha colloidal gold curves within normal limits after treatment Th first and midzone colloidal gold curves became negative (nearly so one to two or more years after treatment Usuall the return to normal conditions was more rapid and defini

than the reduction in the positivity of the quantitative Kolmer test on spinal fluid

BLOOD.—Insufficient quantitative serologic tests of the blood were made to evaluate the response of these tests following treatment. A study of the qualitative results in four routine tests (Kolmer, Kahn, Hinton and Kline) did not indicate a striking effect on the serologic reactions of the blood within the limits of our period of observation, although a reduction in the positivity of one or more of the tests did occur frequently. However, this is not considered of prognostic significance.

CLINICAL RESULTS.—The response of significant and definite symptoms and of nondegenerative signs was used in evaluating the clinical results of treatment. A sense of improved health, increased well being and gain in weight were noted frequently by both the patient and physician. Of those patients followed for six months to one year, 65 per cent had moderate or great improvement in clinical status. Seventy five per cent of the patients with symptomatic neurosyphilis who were examined for the last time from one to two years after therapy had moderate or great improvement. Eighty six per cent of the patients followed more than two years had similar improvement (Table 6). Disregarding the length of observation after treatment and the diagnostic categories, 70 per cent of the patients had improved moderately or greatly, 27 per cent had received no symptomatic benefit or were worse than before treatment, and 3 per cent had obtained slight benefit.

Thirteen patients showed no clinical improvement (Table 6), but 5 were without complaints before treatment. Hence no subjective clinical improvement was expected in these 5 patients. They are included, nevertheless, in the calculation of percentage showing no improvement.

Thirteen patients who had asymptomatic neurosyphilis remained asymptomatic throughout observation and data on them are not included in Table 6. Two patients with taboparesis and convulsive seizures were not benefited as far as the seizures were concerned. One patient with prenatal asymptomatic neurosyphilis and active interstitial keratitis showed slight improvement in the symptoms of keratitis. Two patients with gastric crises were not benefited by combined therapy or further treatment with penicillin. Three patients with optic atrophy had apparent arrest of the condition, while 2 had some restoration of vision and 2 had progression of loss of vision.

TABLE 6

Clinical Response of 63 Patients Following Malaria penicillin Before Further Therapy

Time of examination and result	Diagnosis					Total
	Paresis	Tabo-paresis	Tabes dorsalis	Primary optic atrophy	Meningo-vascular neuro-syphilis	
6 mo—1 yr						
Moderate or great improvement	6	9	5	2	4	26
Slight improvement	1	0	1	0	0	2
No improvement	2	0	2	3	1	8
Worse	1	0	0	2	1	4
1—2 yr						
Moderate or great improvement	4	4	2	0	2	12
Slight improvement	0	0	0	0	0	0
No improvement	1	1	2	0	0	4
Worse	0	0	0	0	0	0
More than 2 yr						
Moderate or great improvement	4	1	1	0	0	6
Slight improvement	0	0	0	0	0	0
No improvement	0	1	0	0	0	1
Worse	0	0	0	0	0	0
Over all summary						
Moderate or great improvement	14(74%)	14(83%)	8(62%)	2(29%)	6(75%)	44(70%)
Slight improvement	1	0	1	0	0	2
No improvement	3	2	4	3	1	13**
Worse	1	0	0	2*	1	4

* One patient had chorioretinitis and optic atrophy.
** Five patients were initially subjectively asymptomatic

There were 2 patients with paresis and 2 with tabes dorsalis, with the arrested type of spinal fluid before treatment One patient with paresis and 1 with tabes dorsalis received little clinical benefit and they were given further therapy The other 2 patients remained subjectively asymptomatic Three of the 4 patients had a decrease in the titer of the Kolmer spinal fluid test following treatment, the cell count and total protein remaining normal in all

The duration of symptoms of neurosyphilis before treatment was one year or less in approximately 33 per cent of the patients and more than one year in about 67 per cent The majority of patients with taboparesis and tabes dorsalis had symptoms for more than one year Patients with meningovascular neurosyphilis usually had symptoms less than one year, while the duration of symptoms of those with paresis and primary optic atrophy was equally divided within these periods The same proportion of good clinical results were noted in patients with symptoms of more than one year's duration, as compared to those patients with symptoms of less than one year's duration

Further treatment of some type was given to 22 patients (29 per cent), and 74 per cent of these 22 patients received more penicillin Five patients received more chemotherapy, while 17 had 4 000 000 to 6,000,000 additional units of penicillin One of these 17 patients received cabinet fever therapy also He had progressive paresis and in spite of treatment with fever and penicillin his condition deteriorated gradually Twenty of the patients had further therapy recommended at six months and the remainder at twelve months Further therapy was advised because of incomplete symptomatic improvement in 12 cases In 4 cases additional treatment was given because the cell count was more than 5 per cubic millimeter In 3 cases further therapy was given because of a combination of elevated cell count and incomplete syphilitic benefit insurance (Table 7)

Three patients with tabes dorsalis and 1 with paresis were either worse or no better after further treatment Three patients with taboparesis, 2 with paresis, 1 with tabes dorsalis and a patient with cervical pachymeningitis (meningovascular syphilis) were improved The chief supplemental treatment was penicillin, although a few patients received chemotherapy and 2 were trated in a fever therapy cabinet The spinal fluid examinations showed essentially no change in those with paresis and taboparesis (arrested type after combined treatment) One patient with tabes

dorsalis showed improvement in the fluid, while another tabetic patient had a more abnormal cell count, although symptomatically improved, and 2 were essentially unchanged (Table 7)

EFFECTS OF VARYING AMOUNTS AND TYPES OF PENICILLIN — The records were reviewed to determine the effect of total doses of less than 4,000,000 units of penicillin with the course of malaria Nine of the 14 patients had good clinical results These included 4 patients who had paresis, 4 who had taboparesis and 1 who had tabes dorsalis Of the 4 patients who had poor clinical

TABLE 7

Further Treatment Given

Further Treatment	Diagnosis						Total
	Paresis	Taboparesis	Tabes dorsalis	Primary optic atrophy	Meningovascular neurosyphilis	Asymptomatic neurosyphilis	
Cases total	5	5	4	2	2	4	22
Reasons							
Incomplete symptomatic benefit	3*	5*	2	2*	2	1**	15†
More than 5 cells cu mm	3*	1*	1	1*	0	1	7†
Insurance	0	0	1	0	0	2	3
Clinical response							
Improvement	2	3	1	0	1	0	7
No change	0	0	3	0	0	0	3
Worse	1	0	0	0	0	0	1
Response of cerebrospinal fluid							
Improvement	0	0	1	0	0	0	1
No change	3	3	2	0	0	1	9
Worse	0	0	1	0	1	0	2

* One patient had both [illegible] complete symptomatic benefit and high cell count in the [illegible]

** [illegible]

† [illegible] c bene-

results 3 had paresis and 1, a child, had asymptomatic neurosyphilis and interstitial keratitis Eleven of the 14 patients had definite improvement in the condition of the spinal fluid, while 3 patients, 2 with paresis and an asymptomatic patient, had active, unchanged or more abnormal fluids after therapy Five patients receiving penicillin in beeswax and peanut oil with concurrent malaria had a satisfactory clinical result and the spinal fluids were definitely improved However, the spinal fluid of one patient with tabes dorsalis remained active

Four to six million units of crystalline penicillin G with malaria were given to 27 patients The majority of these patients were followed one year or less The number of patients is small, but the results do not indicate a greater efficacy in this type of penicillin in combination with malaria An over all 67 per cent of the patients had a good clinical result, while 78 per cent had low grade active or inactive abnormality of the spinal fluid Patients with optic atrophy had the poorest clinical results, as with other methods of treatment, and those with tabes dorsalis had the next poorest

EFFECTS OF PREVIOUS CHEMOTHERAPY —The results obtained for the 30 patients who received twenty treatments with arsenic and twenty with heavy metal before combined treatment (Table 3) were compared with the results for those who had no previous therapy Although the number of patients is too small for more than a suggestive trend the results were practically identical Twenty per cent in each group had poor clinical results and 80 per cent had satisfactory clinical progress In each group were 4 patients who did not have arrested fluids following treatment with malaria and penicillin The number of patients is too small to evaluate the results in the individual diagnostic categories However, it appears that there was no significant difference in the clinical or spinal fluid response to treatment in the various types of neurosyphilis in these two groups

SUMMARY AND CONCLUSIONS

Seventy six patients with generally severe neurosyphilis who received a combination of malaria and penicillin therapy have been studied The findings represent the results achieved from a single combined course of treatment Thirteen patients had asymptomatic neurosyphilis

The over all summary of the spinal fluid examinations reveals that 53 per cent of the 76 patients had group III fluids

before treatment, while 1 per cent were classified similarly when last examined Arrested disease in the spinal fluids was found in 5 per cent of the patients before malaria-penicillin treatment, while 63 per cent of the patients had similar findings after treatment Eighty-eight percent of the patients had minimal (group I) abnormalities, arrested condition or normal fluids following treatment No striking difference in the response of the spinal fluid abnormalities in the various types of neurosyphilis could be noted, although in general it can be said that a higher percentage of paretics had abnormal tests following treatment than any other group Four per cent of the patients had a relapse in the cell count after an initial return to normal

The clinical response was satisfactory in 70 per cent of the 63 patients who had symptomatic neurosyphilis before treatment and 6 per cent of the 63 patients were clinically worse than before treatment Only 29 per cent of the patients with symptomatic optic atrophy had some improvement of vision, while 80 per cent of the patients with symptomatic paresis and taboparesis had satisfactory clinical responses The lapse of time seemed to improve the clinical results, since 65 per cent of the patients had moderate or great improvement at six months to one year, as compared to 86 per cent when last examined more than two years after treatment Part of this apparent improvement with longer period of observation after treatment is due to the selection of some cases earlier for further treatment because of incomplete response to initial therapy

The number of patients available to evaluate the results of further therapy was small, although not all patients were benefited by more treatment A small group of patients receiving crystalline penicillin G combined with malaria did not show a significant difference in improvement, clinically or in results of the spinal fluid examinations from those who received sodium penicillin with malaria The clinical and spinal fluid responses of patients who had had chemotherapy before combined malaria and penicillin treatment were comparable to those of patients who were previously untreated

From a review of the literature and our own experience, it is believed that a combination of malaria and penicillin is the treatment of choice in severe parenchymatous types of neurosyphilis Patients with the milder forms of neurosyphilis, such as asymptomatic and meningeal syphilis, may first be given the advantage of one or two courses of penicillin alone If the reactions

of the spinal fluid do not become normal or approach normal, and if the clinical findings do not improve, combined therapy then should be administered

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TREATMENT OF NEUROSYPHILIS WITH PENICILLIN ALONE AT BELLEVUE HOSPITAL

By

*Bernhard Dattner**

Since April, 1944, approximately 400 patients with various forms of neurosyphilis were treated at Bellevue Hospital exclusively with intramuscular injections of penicillin The present report, however, comprises only 376 patients They were selected because their spinal fluid findings before treatment indicated activity of the syphilitic process, i e, lymphocytosis and increased total protein values Previous experience in the treatment of patients with neurosyphilis has shown that changes in the abnormal spinal fluid following treatment offer a more reliable criterion of therapeutic efficacy or failure than clinical signs and symptoms

Of the 376 patients with "active spinal fluids" 67 were lost and 8 are known to have died within 6 months following the end of treatment This represents a loss of 20 per cent The remaining 301 were followed up for more than 6 months Ten per cent of the 301 patients have now been observed for more than 3 years, 35 per cent for more than 2 years and 70 per cent for more than one year The longest observation time was 45 months

The first table specifies the diagnostic groups and the length of observation of each group in months

All observers who have reported the effect of penicillin on the spinal fluid syndrome agree that the first abnormal finding of the spinal fluid to become normal is the cell count The protein

* Rapid Treatment Center—Bellevue Hospital and New York University

TABLE I

Length of Observation Time of Patients With Active Neurosyphilis Treated With Penicillin

Diagnosis	Number of Patients	Months			
		6-12	12-24	24-36	More than 36
Asymptomatic	61	25	20	15	1
Meningovascular	75	27	27	15	6
Tabes dorsalis	83	22	33	17	11
Dementia paralytica	53	12	12	22	7
Taboparesis	29	4	11	9	5
	301	90	103	78	30

values, the colloidal gold changes and the quantitative complement fixation tests decrease gradually, usually in the order given. Identical observations were made years ago in the follow up of patients who had successful malaria therapy for neurosyphilis. In some patients five or more years passed before the Wassermann reaction and the colloidal gold curve became completely normal. This holds true for all types of neurosyphilis. In their last paper, Stokes and co-workers reported that they were impressed by the high proportion of patients showing improvement in spinal fluid abnormalities regardless of whether they were treated for asymptomatic neurosyphilis or general paresis. This must obviously be so because in all types of neurosyphilis the response to therapy as reflected in the spinal fluid findings is the direct result of the effect of treatment upon the spirochetes and not upon the structural changes due to the infection and the subsequent clinical manifestations.

The gradual reversal of the pathologic spinal fluid findings to normal seems to indicate the arrest of the pathologic process and, therefore, is of favorable prognostic significance. There are many reasons for this belief. It is a well known fact that the most severe form of neurosyphilis, general paresis, if untreated, has a constant and characteristic spinal fluid syndrome. It is also notorious that the older ineffective methods of treatment failed to change the spinal fluid findings of paretics and that only after the introduction of malaria therapy was the gradual reversal of the abnormal spinal fluid findings noted. After quantitative values of great accuracy became available for the complement fixation and colloidal gold tests, it became even

CASE I

Penicillin Success After Malaria Failure

F J E. 50 W M General Paresis

Primary Age 40 Three Years Routine Treatment

Test No	Date	Blood Wass	S F Wass	Colloidal Gold	T P	Pandy	Cells
1	7 16 43	4+	4+	5555	55	4+	140/3
August 1943-Tertian Malaria (5), Typhoid (3) and Mapharsen (10)							
2	11 30 43	4+	4+	2211	85	4+	800/3
January 1944—20 Pentavalent Arsenicals							
3	3 16 44	4+	4+	5554	62	3+	70/3
April 1944—20 Additional Pentavalent Arsenicals							
4	5 22 44	4+	4+	No Data	60	2+	26/3
5	10 4 44	4+	4+	No Data	62	2+	250/3
October 1944—4 Million Units Penicillin							
6	10 20 44	4+	150	164	46	2+	43/3
7	11 20 44	76	110	154	47	1+	36/3
8	1 15 45	44	71	144	37	F T	9/3
9	4 10 45	39	80	122	37	V F T	14/3
10	7 11 45	20	77	130	38	V F T	3/3
11	10 29 45	21	70	133	34	V F T	6/3
12	1 18 46	17	41	68	33	V F T	6/3
13	7 5 46	11	34	72	32	V F T	3/3
14	10 25 46	10	37	79	28	V F T	6/3
15	5 16 47	10	28	75	27	±	8/3
16	11 28 47	5	17	71	29	±	8/3

THE TREATMENT OF LATE SYMPTOMATIC NEURO-SYPHILIS AT THE BOSTON PSYCHOPATHIC HOSPITAL

A STUDY OF THE RESULTS OF PENICILLIN THERAPY IN 394 PATIENTS TREATED BETWEEN FEBRUARY, 1944 AND MARCH, 1948*

By

Israel Kopp, Augustus S Rose, and *Harry C Solomon*

A total of 394 patients with neurosyphilis have received penicillin therapy at the Boston Psychopathic Hospital from February 1, 1944 to March 1, 1948 (Table I) Thirty-two patients (8%) have died (Table II) Death was due to syphilis in one-half of this group, a mortality rate of 4% Twenty-four patients (6%) have been lost to clinical follow-up, most of these having lapsed during the past year A total of 339 patients remain under observation

In the present communication we wish to present a summary of our experiences with penicillin in the treatment of neurosyphilis Since the greater portion of our patients are of the paretic variety, we shall concern ourselves especially with this group at this time

MATERIAL

Of the entire group 305 patients (77%) were classified as paretic These patients were diagnosed as general paresis, tabo-paresis, and juvenile paresis (Table I) Symptoms of mental disorder of varying degrees were present Eighty per cent of this group (245 patients) were psychotic The various types of psychoses present are shown in Table III Of particular interest is the fact that 55 patients (18%) of the entire group of patients presented a schizophrenic type of syndrome

As has been indicated in previous reports, many of the patients had been hospitalized in other mental institutions and had received chemotherapy prior to admission to the Boston Psychopathic Hospital When treatment was completed and if clinical improvement was sufficient, the patients were followed in the out-patient department Otherwise, the patients were transferred to the Metropolitan State Hospital nearby, and were seen at repeated intervals When necessary, patients were readmitted to the Boston Psychopathic Hospital for additional therapy

* This work was begun under contract with the Committee on Medical Research of the Office of Scientific Research and Development later continued under grants in aid from the United States Public Health Service

TABLE I

Total Patients Treated with Penicillin February, 1944 to February 1948

Diagnosis	Number
General Paresis	292
Juvenile Paresis	13
Tabes	37
Primary Optic Atrophy	14
Meningo vascular Neurosyphilis	21
Asymptomatic Neurosyphilis	17
Total	394

METHODS OF TREATMENT

Combined therapy consisting of penicillin and fever has been administered to most of our patients Fever was given in amounts equivalent to one half of the usually accepted course of therapy, 5 to 7 paroxysms of malaria with temperature above 104° (R) or 20 hours of artificial fever above 105° (R)

Penicillin was given before, during or after fever Penicillin alone was administered to 94 patients and penicillin combined with fever to 300 patients (Table IV) When retreatment was necessary, an additional course of penicillin usually equivalent to the original course was given In some instances, more so when penicillin alone had first been used, fever was added

The dosage of penicillin first used was 3 million units Experience with 100 cases given this dosage combined with fever revealed the need for retreatment in more than one third of the cases followed one year or more The penicillin dosage was then increased to 6 million units in July, 1945 During the past 18 months penicillin G has been used

The criteria for retreatment remain as previously stated These are clinical or spinal fluid relapse, and failure to show significant improvement in the spinal fluid within 3 6 months The cell count should fall to normal within 3-6 months, and the

TABLE II

The Number of Deaths in 394 Penicillin treated Cases of Neurosyphilis

	No	Due to G P
Within 3 mos of treatment	13	4
Within 6 mos of treatment	6	4
Within 12 mos of treatment	4	3
Within 24 mos of treatment	8	5
Within 36 mos of treatment	1	
Total	32	16

TABLE III

Diagnostic Subdivisions of Paretic Patients

I With Psychosis		
1 Simple dementia	140	(46%)
2 Grandiose	25	
3 Manic depressive	23	
4 Schizoid	55	(18%)
5 Psychoneurosis	2	
II Without Psychosis	60	(20%)
Total	305	

TABLE IV

Modes of Penicillin Therapy vs Diagnostic Groups

	Penicillin Alone	Penicillin Plus Malaria	Penicillin Plus Cabinet	Penicillin Plus Malaria and Cabinet	Other Types
General paresis, Tabo-paresis, Juvenile paresis	60	203	34	4	4
Tabes	8	12	17		
Optic atrophy	3	7	4		
Meningo vascular	15	2	4		
Asymptomatic	8	7	2		
Total	94	231	61	4	4

total protein should show considerable improvement within 6 to 9 months

The different modes of therapy for the various diagnostic groups of neurosyphilis are seen in Table IV. Patients given both malaria and fever cabinet therapy were those whose malarial fever had spontaneously ceased and were then given artificial fever, or patients who tolerated treatments in the fever cabinet so poorly that malaria was substituted

Clinical Results

An evaluation of the clinical status of 305 paretic patients followed 3 months or more after penicillin therapy reveals that 170 (56%) are improved, the majority able to reside in the community (Table V) One hundred and four patients (34%) are unimproved but a fair number of these are able to remain in the community Thirty-one paretic patients have died but in only 16 can death be attributed to the syphilitic process

Our findings also reveal that clinical improvement continues during the first year to a maximum of approximately 70% (Table VI) However, little additional improvement occurs

TABLE V

Clinical Status of 305 Penicillin treated Paretic Patients Followed 3 Months or More (March 1948)

Clinical Status	No Patients
Improved	170 (56%)
Unimproved	104 (34%)
Dead	31 (10%)
Total	305(100%)

thereafter so that if improvement is to occur, it will usually be apparent within at least one year after combined penicillin and fever treatment The smaller percentage of patients in the group observed for three years or more (Table VI) can be attributed to the fact that in this group there were a large number of individuals who had been transferred to the Boston Psychopathic Hospital for treatment after a long period of hospitalization elsewhere The increase in the percentage of improvement (65 to 85%) from the first to the second year when 6 million units of penicillin were used is rather striking, but may be due in part to the fact that there were more early cases in the group of this year

The importance of the original clinical picture presented by the patient in the therapeutic results obtained is seen in Table VII Of 59 paretic patients without psychosis, 50 (85%) improved with combined penicillin and fever therapy Of 211 psychotic patients only 117 (55%) improved That the type of psychosis present before therapy is also of considerable importance in the results obtained is seen in Table VII Thus, only 25% of 48 patients with schizophrenic type of psychosis improved, in sharp contrast to 60%, 65% and 90% of improvement in the simple dementia, grandiose and manic-depressive types respectively

We have attempted to evaluate different schedules of penicillin therapy for paresis Three million units of penicillin given in 10 days or less with fever resulted in improvement in 58% of 79 patents (Table VIII) When this same dosage of penicillin was given over a 15-day period, 71% of 52 patients improved That the time factor may be of greater importance than the absolute amount of penicillin given is indicated by a comparable degree of clinical improvement, approximately 70%, when 3 or 6 million units of penicillin were given over a 15-day period

TABLE VI

Clinical Status of [illegible] Cases of General Paresis Treated with Penicillin *

Schedule Penicillin	Observation Period														
	12 to 2[illegible] mos					24 to 35 mos					35 to 45 mos				
	Tot	Imp	Unimp	Worse	Dead	Tot	Imp	Unimp	Worse	Dead	Tot	Imp	Unimp	Worse	Dead
3 Million U 5 to 10 days	10	6 ([illegible]0%)	4	0	0	33	20 (60%)	11	1	1	36	20 (56%)	13	3	0
3 Million U 15 days	30	23 (77%)	5	0	2	20	14 (70%)	4	1	1	2	0	1	1	0
6 Million U 15 days	[illegible]2	34 (6[illegible]%)	13	2	3	21	18 (8[illegible]%)	2	0	1	1	1	0	0	0
Totals	92	6[illegible] ([illegible]%)	22	2	[illegible]	74	52 (70%)	17	2	3	39	21 (54%)	14	4	0

* With and without fever

TABLE VII

The Clinical Status of 270 Living Paretic Patients vs Original Clinical Picture (Followed 3 Months or More)

	Improved	Unimproved	Total
Without psychosis	50(85%)	9	59
Simple dementia	76(60%)	50	126
Schizoid	12(25%)	36	48
Grandiose	11(65%)	6	17
Manic depressive	18(90%)	2	20
Total	167(62%)	103	270

TABLE VIII

Clinical Status of 205 Penicillin treated Paretic Patients Followed From 12 to 45 Months vs Mode of Penicillin Therapy (January 1948)

Schedule of Penicillin Treatment	No Cases	Clinically Improved
3 Million Units 5 to 10 days	79	46(58%)
3 Million Units 15 days	52	37(71%)
6 Million Units 15 days	74	53(72%)
Total	205	136(67%)

Fifty four of 60 patients to whom penicillin alone had been given have been followed for one year or more Retreatment was necessary in 28 patients (52%) because of evidence of relapse or inadequate improvement in symptoms or spinal fluid (Table IX) Eleven (40%) of this latter group were treated with fever as in our opinion penicillin alone was not giving optimal results Of the 219 patients treated with combined fever and penicillin only 45 (21%) were retreated The present clinical status of the entire retreated group parallels that of the whole series

Table IX reveals also the importance of the dosage of penicillin when combined with fever in the treatment of general

TABLE IX

Retreatment in Paresis

(Patients Followed 1 Year or More to March 1948)

Schedule of Penicillin Treatment	No Patients	Retreatment			Total
		Penicillin	Fever	Chemo-therapy	
3 Million U	31	10	8	2	20(6[illegible]%)
6 Million U	19	5	3	—	8(42%)
6 Million U G	4				—
Total	54	15	11	2	28(52%)
Plus fever					
3 Million U	121	21	8	2	31(26%)
6 Million U	73	6	4	—	10(14%)
6 Million U G	25	4	—	—	4(16%)
Total	219	31	12	2	45(21%)

paresis The table is based on patients observed for one year or more Retreatment was more frequent when only 3 million units of penicillin were used even if fever was administered

Spinal Fluid Results

The changes in the various elements of the spinal fluid are shown in Charts I II III and IV The number of cells in the spinal fluid falls rapidly as a result of treatment so that 73% of the patients reveal a normal cell count within 3 months Of considerable interest is the fact that the maximum number of normal cell counts is obtained in 18 months and that this value remains fairly constant up to 36 months

The total protein values show also a progressive increase in the number of normal values As with the cell count the maximum number of normal values is obtained at about 18 months and remains fairly constant thereafter

The gold sol shows a progressive fall It is our impression that gold sol values are more rapidly reduced and normal readings more frequently obtained with penicillin than with former methods of treatment

The strength of the Wassermann reaction diminishes progressively as a result of therapy more rapidly during the first 9 months less so from 9 to 18 months and tapering off thereafter

In Charts III and IV are shown the comparative effects of the two different schedules of penicillin treatment 3 and 6 mil

TABLE X

Status of Last Spinal Fluid Obtained on 188 Cases of Pares s 12 to 45 Months After Penicillin Therapy With or Without Fever (January, 1948)

Schedule of Penic ll n Therapy	Sp nal Flu d Grouping *				
	Neg	I	II	III	Total
3 Mill on U 5 10 days	5	9	53 (70%)	9	76
	19%				
3 Mill on U 15 days	1	5	31 (70%)	7	44
	14%				
6 M ll on U 15 days	4	11	42 (62%)	11	68
	22%				

* According to cooperative cl nic groups

CHART I

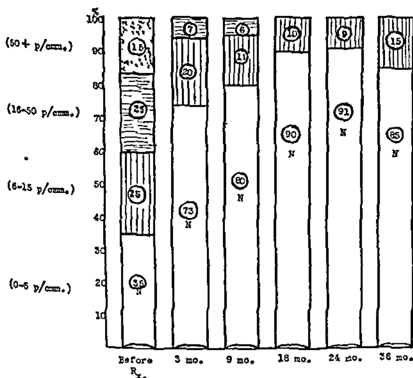
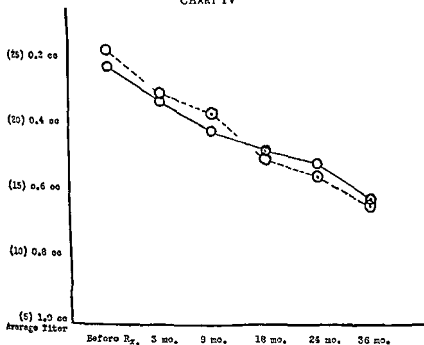


CHART II

CHART III

CHART IV



lion units, upon the Wassermann titre and gold sol The curves are nearly identical and can practically be superimposed Similar findings were obtained with the cell counts and the total proteins

The status of the last spinal fluid as of December, 1947 obtained on 188 cases of paresis from 12 to 45 months after penicillin treatment is shown in Table X There is little significant difference between the various modes of penicillin therapy

COMMENT

Our data support the contention that a short course of malaria when added to penicillin given over a 15 day period is equal to or superior to any treatment thus far known in late symptomatic neurosyphilis Our findings also indicate that the time interval of penicillin administration is of importance, those patients receiving penicillin over a 15 day period doing better than those who received a similar dosage in 10 days or less At the present time we are unable to state that a 6 million unit dosage of penicillin is superior to that of 3 million However, it appears that retreatment is less necessary when 6 million units of penicillin G are used The less frequent necessity for retreatment in those patients who received combined penicillin and fever therapy

points to this form of therapy as the most desirable at this time

Our findings also reveal the importance of the type of paretic syndrome which is treated The therapeutic results will depend not only on whether one is dealing with psychotic or non psychotic patients, but also on the type of psychosis which is present These factors, though well known prior to penicillin therapy, have not been sufficiently stressed when the results of various clinic groups have been compared They may well be responsible for the differences obtained

Summary and Conclusions

1 Three hundred and ninety-four patients with neurosyphilis have been treated with penicillin from February 1, 1944 to March 1, 1948 Three-fourths of the patients were of the paretic variety

2 Penicillin was given in 3 or 6 million unit dosages over intervals of 5 to 15 days The great majority of the patients were also given fever, either malaria or fever cabinet in approximately one half of the usually accepted course

3 Thirty-two of the patients have died, but in only 16 can death be attributed to the syphilitic process The adjusted mortality rate is 4% Twenty-four patients (6%) have been lost to clinical follow up

4 Of 270 paretic patients followed 3 months or more 62% have improved The clinical syndrome is of considerable importance in the therapeutic results obtained Of 59 non psychotic paretic patients, 85% have improved Of 211 psychotic patients 53% have improved The type of psychosis also plays an important role in the therapeutic results obtained, improvement ranging from 25% in 48 patients with schizophrenic-like syndromes to 90% in those with manic depressive types These factors must be taken into consideration when comparing the results of treatment from different clinic groups

5 Penicillin administered over a 15-day period and combined with fever gave better clinical results than when given for 10 days or less Clinical improvement resulted in 71% of 52 patients in the former category and in 58% of 79 patients in the latter No significant difference was found when 3 or 6 million units of penicillin were used

6 Retreatment was more frequent when penicillin alone was used, 52% of 54 patients, in contrast to 21% of 219 patients to whom combined fever and penicillin was given Retreatment

was more frequent when 3 million units of penicillin were used with or without fever

7 The spinal fluid cell count falls rapidly as a result of combined penicillin and fever therapy, three quarters of the fluids revealing a normal cell count within 3 months' time Approximately three fourths of the protein values become normal at the end of one year The maximum number of normal values of the cell count and protein is reached in about 18 months' time Gold sol values show a progressive decline and become negative more frequently than with other methods of therapy Nineteen per cent of 188 fluids obtained 12 to 45 months after therapy were negative or nearly so

8 The changes in the spinal fluid following 3 and 6 million units of penicillin are practically identical

9 A short course of malarial therapy when added to penicillin given over a 15 day period is to the present time and in our experience equal to or superior to any treatment thus far known in late symptomatic neurosyphilis

TREATMENT OF NEUROSYPHILIS AT HOT SPRINGS V D MEDICAL CENTER

By
*George E Parkhurst**

This study presents the progress, as measured by the spinal fluid examination, among neurosyphilis patients treated with mechanical hyperthermy, malaria, or penicillin, at the Hot Springs Medical Center, Arkansas All of the 458 cases included in this study had a minimum observation period of six months Excluded from the study was any early neurosyphilis case having manifestations of primary or secondary syphilis or any patient who had had fever therapy within one year prior to treatment being evaluated

The hyperthermy series is composed of 122 patients, most of whom were treated during 1943 and 1944 This therapy comprised 30 to 60 hours of fever above 104° F in a Kettering hypertherm (83 per cent of these patients received 40 to 50 hours about 104° F)

The quartan malaria series consisted of 196 patients, most of whom were treated during 1944 and 1945 Sixty eight per cent of the patients experienced 40 to 50 hours of fever above 104° F ,

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15 per cent experienced fewer hours, and 17 per cent more hours above 104° F

The penicillin series consisted of 140 patients treated between June 1945 and March 1946 Total dosage was 6,000,000 units of amorphous penicillin in peanut oil and beeswax (400,000 units every 24 hours for 15 days) plus 8 injections of arsenoxide and 5 injections of bismuth given concurrently.

Because the majority of cases in all groups had reported wide variation in amount and type of previous arsenical and bismuth therapy, and because so little of it could be verified, no endeavor has been made to classify the results of this study by either amount or type of previous treatment The patients treated with the hypertherm and with malaria were discharged with the recommendation that additional arsenical and bismuth therapy be given at the local health department, but data are not available on the amount of treatment, if any, which was given after discharge from the Medical Center No additional therapy was recommended for the patients treated with the penicillin schedule

In Table 1 cumulative failure rates after 24 months posttreatment observation are shown by type of neurosyphilis and method of treatment The cases were divided into three groups (1) asymptomatic neurosyphilis, early and late, (2) vascular and meningovascular neurosyphilis and (3) paresis, tabes dorsalis, and taboparesis The failure rate was calculated by adjusting for lapses from observation, on the assumption that the same proportion of failures occurred among the cases lapsing as among those who remained under observation

There were no significant differences in failure rates among the three types of treatment in any diagnostic group Where a small numbr of cases was observed, as occurred in the later observation periods of several series, one failure would cause a great increase in the cumulative failure rate Since the distribution of cases by type of neurosyphilis is approximately the same for mechanical hyperthermy, malaria and penicillin, the 'total all diagnoses' which eliminates small totals, presents the best indication for comparing failure rates by type of treatment By the end of 24 months posttreatment observation, 11 6 per cent of the patients treated with the penicillin schedule had failed as compared with 12 6 per cent following malaria and 14 3 per cent following mechanical hyperthermy On the basis of failure rates penicillin appears equally as effective as the hypertherm or malaria in the treatment of neurosyphilis

TABLE I

Classification of Failures by Type of Neurosyphilis and Method of Treatment

Type of Neurosyphilis	Method of treatment	Total cases treated	Total cases followed 24 months	Failures by End of 24 Months Observation				
				Clinical failure only	Spinal fluid failure only	Both clinical and spinal fluid failure	Total failure	Cumulative failure rate* (Percent)
Asymptomatic (Early and Late)	Penicillin	68	16	0	3	0	3	9.3
	Malaria	102	43	1	4	1	6	11.3
	Hypertherm	63	32	1	2	0	3	6.2
Vascular and Meningovascular	Penicillin	14	7	1	1	0	2	16.4
	Malaria	21	4	2	0	0	2	34.4
	Hypertherm	15	6	0	1	1	2	22.1
Paresis Tabes dorsalis and taboparesis	Penicillin	58	0	1	4	3	8	14.7
	Malaria	73	9	2	1	3	6	12.0
	Hypertherm	44	21	1	1	6	8	23.0
Total all Diagnoses	*Penicillin*	*140*	*53*	*2*	*8*	*3*	*13*	*11.6*
	Malaria	196	86	5	5	4	14	12.6
	Hypertherm	122	59	2	4	7	13	14.3

* The failure rate is calculated by adjusting for lapses assuming that the same proportion of failures would have occurred among the cases lapsing from observation as among those who remained under observation, and cannot be calculated from data presented in this table

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CHART 1

PERCENTAGE OF CASES SHOWING ACTIVITY* IN THE SPINAL FLUID BY TYPE OF TREATMENT AND PERIOD OF POST TREATMENT OBSERVATION

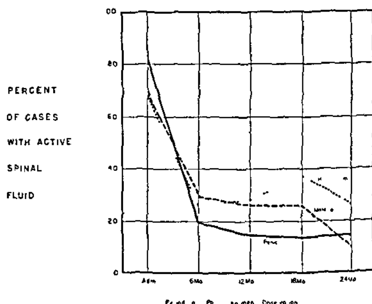


Chart 1 presents the per cent of cases showing activity in the spinal fluid at the time of admission and at six-month intervals for two years following treatment An active spinal fluid is defined as one in which the cell count is greater than 4 and/or the total protein is greater than 30 mg per cent Cases that are re treated for either clinical or spinal fluid failure, or both, are considered active in subsequent periods However, only a proportion of these cases is included in subsequent periods (the proportion varies with the percentage of total cases examined in a particular period) Sixty nine per cent of the cases in the mechanical hyperthermy and in the malaria series and 83 per cent of the cases in the penicillin series showed activity in the spinal fluid on admission By 24 months following treatment, 10 per cent of the malaria treated patients, 15 per cent of the penicillin-treated patients, and 26 per cent of the hypertherm treated patients still showed activity in the spinal fluid Chart 1 indicates that penicillin eliminates activity in the spinal fluid more rapidly than does malaria or hypertherm treatment

A third comparison of penicillin, malaria, and mechanical

hyperthermy is based on changes in spinal fluid groupings following each type of treatment This is mainly a comparison of the response of the Kolmer test on spinal fluid by the methods of treatment since grouping is usually dependent upon the degree of Kolmer positivity in dilutions and may not have correlation with activity In defining the groupings of spinal fluids, the following criteria were used:

Group III—Positivity (4+ or 3+) in the Kolmer complement fixation test in 125 cc of fluid (443), or greater, usually with markedly increased protein and/or cells

Group II —Kolmer positive from 5 cc to 25 cc (4 or 44) of spinal fluid, usually with moderately increased protein and/or cells

Group I —Those having a cell count of more than 4 and/or elevated protein above 30 mg per cent and having a negative Kolmer complement fixation

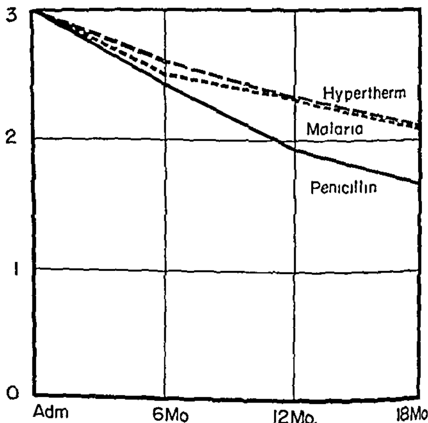
Negative —Those normal in all elements

Upon admission to treatment, 72 per cent of the patients had a group III spinal fluid The following chart applies only to these patients Essentially it is a showing of weighted averages, which were derived by assigning weights of 3, 2, 1, and 0 to the number of fluids in Groups III, II, I, and Negative, respectively, in each of the observation periods Thus, since only Group III patients were used, this chart shows a value of 3 0 on admission and, if in any one therapy they had all reached negativity by 18 months, that value would have been zero Reference to this chart indicates that there is little difference between malaria and mechanical hyperthermy in their respective effect on the spinal fluid, but that penicillin generally brings about more rapid and greater changes than the other two methods of treatment If this grouping of the spinal fluid is used as an indication of improvement in neurosyphilis, it would then appear that penicillin is the most effective of the three types of therapy

Table 2 presents in summary form the results of spinal fluid tests of patients treated with penicillin at various periods of observation It shows the per cent of cases which are abnormal in each period for each of the four elements of the spinal fluid syndrome Limits, as indicated in the table, were established for each of the elements to determine abnormality The percentage of cases which were abnormal in each of the observation

CHART 2

COMPOSITE SPINAL FLUID GROUP CHANGE FOLLOWING TREATMENT OF PATIENTS HAVING GROUP III SPINAL FLUID ON ADMISSION



periods follow the expected pattern—the cell count returning to normal first, followed by total protein, colloidal gold, and Kolmer Wassermann in that order

The response to treatment among patients with early asymptomatic neurosyphilis (under 4 years) was significantly (at 1% level) more rapid than among late asymptomatic patients Of the 24 cases observed for 12 to 24 months, 20, or 83 3 per cent showed a negative spinal fluid test on last observation in that period Among the late asymptomatic neurosyphilis cases, 46 or 31 7 per cent, of the 145 cases observed between 12 and 24 months had a negative spinal fluid on last observation in that interval

TABLE 2

Course of *Cerebrospinal* Fluid Abnormalities in Total Neurosyphilis Cases Treated *with Penicillin* Therapy

Cerebrospinal Fluid Abnormality		*Admission*	Post treatment observation period (months)			
			6	9–12	15–18	21–24
Cells	Number observed	139	119	100	70	47
	Number abnormal (Greater than 4)	104	9	4	0	1
	Percent abnormal	74.8	7.6	4.0	0.0	2.1
Total protein	Number observed	140	120	99	71	46
	Number *abnormal* (Greater than 50 mg %)	80	21	11	6	6
	Percent abnormal	57.1	17.5	11.1	8.5	13.0
Colloidal Gold	Number observed	137	117	99	70	46
	Number abnormal (Greater *than a* 2 reaction)	83	37	26	13	6
	Percent abnormal	60.6	31.6	26.3	18.6	13.0
Kolmer Wassermann	Number observed	140	118	100	71	46
	Number abnormal (Greater than a negative titer)	139	103	79	47	31
	Percent *abnormal*	99.3	87.3	79.0	66.2	67.4

3/16/48

A comparison of the symptomatic cases of neurosyphilis who had had their symptoms for less than one year with those who had had symptoms over one year showed that in general the earlier in the period of symptomatology the case is placed under adequate treatment, the better the response

Summary

(1) On the basis of failure rates, penicillin appears equally as effective as hypertherm and malaria in the treatment of neurosyphilis

(2) Penicillin eliminates activity in the spinal fluid more rapidly than does hypertherm or malaria treatment

(3) By the end of 18 months observation, penicillin treatment reduces the Kolmer test to negativity in a greater per centage of cases than does malaria or hypertherm treatment

NEUROSYPHILIS EVALUATION AFTER TWO YEARS OF TREATMENT WITH PENICILLIN ALONE AND WITH A COMBINATION OF PENICILLIN AND MALARIA

By

Arthur C Curtis, *S F Horne*, and *Dorothy H Norton**

Since 1944 there has been a gradual accumulation of data concerning the effectiveness of penicillin in the treatment of neurosyphilis A report on 118 patients treated in this hospital and observed for a minimum of one year, subsequent to treatment, has been made [1] The present study includes this series of 118 patients who now have been observed for two years subsequent to therapy and also introduces a new one year group This analysis of a larger number of patients and a longer period of observation makes it obvious that the conclusions based on the previous study must be revised

Our first report[1] reviewed the early literature from the first use of penicillin in neurosyphilis by Stokes and his associates[2] and consequently will not be repeated Since then several studies have added to our constantly growing knowledge of the value of penicillin alone and in combination with various other therapeutic agents in the treatment of central nervous system syphilis

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In a three year progress report from the series under study at the University of Pennsylvania, Beerman[3] has noted that penicillin alone is equal to malaria therapy for paresis, and superior to therapeutic malaria for cases of tabo paresis, tabes dorsalis, meningovascular syphilis and asymptomatic neurosyphilis Thus, it seems that the early optimistic reports from these observers are being upheld by longer periods of observation O'Leary and Kierland,[4] conversely, believe that the parenchymatous forms of neurosyphilis are resistant to penicillin therapy and that the 'results from the treatment of neurosyphilis with penicillin are still unpredictable and somewhat erratic"

A number of reports have appeared dealing with penicillin treatment of a small series of patients followed for a relatively short time In general, these papers corroborate the early findings of other workers Jones and Peck[5] treated six patients having dementia paralytica with penicillin alone(two courses, each of 2,400,000 units, separated by one month) and assessed the mental changes by repeated psychometric tests Three patients were improved mentally, one was unchanged and two became progressively worse In all cases there was improvement in the spinal fluid abnormalities Heyman[6] has reported his series of 141 patients with neurosyphilis treated with penicillin (1,200 000 units, later 2,000,000 and 3,000,000 units and finally 5,000,000 units given as 50,000 units every three hours for ten days) and followed for six to nineteen months It is his belief that 4,000,000 units is the minimum satisfactory dosage for the treatment of neurosyphilis The relapse rate with this dosage was approximately 15 per cent and most occurred within six months after therapy When 48 patients treated with 4 000,-000 units of penicillin were compared with a similar number previously treated with fever the response to both types of therapy appeared similar, but symptomatic improvement seemed better with fever He believes that penicillin does not replace fever therapy as the treatment of choice in late symptomatic neurosyphilis Sweitzer[7] reported nine cases of central nervous system syphilis treated with penicillin alone and followed for five to seventeen months Two of these showed negative post-treatment spinal fluid findings and the remainder showed some improvement in spinal fluid abnormalities and in physical well-being Leavitt[8] reported a group of 171 patients with neurosyphilis (73 1 per cent asymptomatic) who were treated with 2 400,-000 units of penicillin or with one of two schedules combining

trivalent arsenic, bismuth and penicillin The group was observed from three to eighteen months after treatment Check up examinations of the spinal fluid showed refinite improvement in 69 6 per cent and normality in 25 1 per cent There was no significant difference in the results obtained from the three methods of treatment

MATERIAL

Since 1944 a total of 539 patients with various types of neurosyphilis have been treated at the University Hospital Four hundred and eight of these patients were treated more than one year ago Eight patients died (none from syphilis) and 145 of these patients did not return for a check up within the one year post treatment period Eighteen patients were removed from the series because they were retreated elsewhere, without apparent reason Seven patients with the inactive spinal fluid formulae of Dattner-Thomas and seven patients who received less or more than 4 000,000 units of penicillin were removed for statistical reasons This report, then, is of 223 patients treated for some type of neurosyphilis who have been observed for a minimum of one year subsequent to treatment One hundred and four of these 223 patients have been followed for at least two years since the termination of treatment

The patients treated were chiefly of the white race there being but four Negroes in this series of 223 people The average age was 37 years the oldest 66 years and the youngest eight years There were 76 females and 147 males

The distribution of the types of neurosyphilis is shown in Table I In spite of an almost equal number of patients treated by the two methods, the two groups are not entirely suitable for statistical comparison In our earlier report,[1] there was a tendency to give fever therapy and penicillin to the more advanced and deteriorated cases However, as the effectiveness of penicillin alone became apparent, we have treated more patients by it alone The two groups are now more nearly equal in severity and rapidity of progression than they were when the first follow up study was made

METHOD OF TREATMENT

All of the patients received either penicillin alone or penicillin plus malaria (Table I) The details of these treatment schedules have been recorded previously[1] but, in summary, the penicillin was administered in saline, 40,000 units intramuscularly every three hours for 100 doses, making a total dosage of

TABLE I

Types of Central Nervous System Syphilis Treated with Penicillin and Malaria and Penicillin

	One Year Group			Two Year Group		
		Treatment			Treatment	
Neurosyphilis	Cases	Penicillin	Penicillin and Malaria	Cases	Penicillin	Penicillin and Malaria
Type	Number	Number	Number	Number	Number	Number
Asymptomatic	26	19	7	8	6	2
Meningovascular	20	12	8	15	9	6
Tabes Dorsalis	63	37	26	25	19	6
Taboparesis	43	17	26	21	8	13
Paresis	71	23	48	35	13	22
Totals	223	108	115	104	55	49

4 000 000 units It was given either alone or in conjunction with therapeutic malaria (tertian) the patient receiving 50 or more hours of fever over 103 5°F rectally

Results—Entire Group

The results of treatment have been most gratifying Tables II and III summarize the results obtained both clinically and in the spinal fluid examinations The response of the cell count, total protein colloidal gold curve and quantitative Kahn serological tests are shown in Figure 1 Recently, in addition, quantitative Kolmer tests have been done and they too follow the same general trend of the quantitative Kahn serological test.

One year after treatment the group of patients who received penicillin alone showed 79 per cent clinical improvement and 82 per cent improvement in the spinal fluid abnormalities The group who received penicillin plus malaria attained 69 per cent clinical improvement and 89 per cent improvement in the spinal fluid findings After two years observation of 104 patients 86 per cent of the penicillin alone group showed clinical improvement and 85 per cent had improvement in the spinal fluid formulae The group that received combined therapy showed improvement respectively, of 66 per cent and 98 per cent Since it was felt that those patients who failed to improve in the year following treatment were in greater danger of deterioration than those who did improve we combined the figures for this group

with those who became worse during the first post treatment year[1] However, those patients who were unchanged during the first year have not shown deterioration during an additional year of observation No patient has shown a progression in the spinal fluid abnormalities during the second post-treatment year The few who have relapsed did so during the first year and have been retreated

At the end of one year 22 (21 5 per cent) of the patients in the penicillin alone group and 14 (12 3 per cent) in the combined

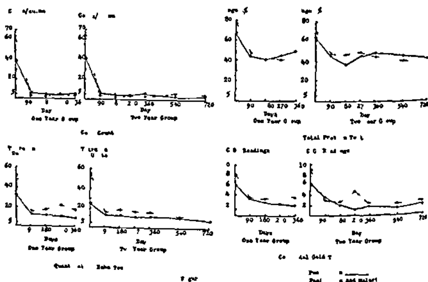


TABLE II

Results of Treatment of 223 Patients Having Several Types of Neurosyphilis With Penicillin and Penicillin Plus Malaria Followed for one Year

Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
Clinical (Asymptomatic omitted)	Penicillin	70	79	17	19	2	2	89
	Penicillin and Malaria	73	69	25	24	8	7	106
Spinal Fluid	Penicillin	89	82	16*	15	3	3	108
	Penicillin and Malaria	102	89	11**	9	2	2	115

* Includes 6 patients with negative spinal fluid before treatment
** Includes 1 patient with negative spinal fluid before treatment

TABLE III

Results of Treatment of 104 Patients Having Several Types of Neurosyphilis With Penicillin and Penicillin Plus Malaria Followed for two Years

Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
Clinical (Asymptomatic omitted)	Penicillin	42	86	6	12	11	2	49
	Penicillin and Malaria	31	66	11	23	5	11	47
Spinal fluid	Penicillin	47	85	8*	15	0	0	55
	Penicillin and Malaria	48	98	1**	2	0	0	49

* Includes 4 patients with negative spinal fluid before treatment
** This patient had negative spinal fluid before treatment

group had negative spinal fluid examinations During the second year seven patients who had received penicillin alone developed negative spinal fluid examinations In the combined therapy group, three reverted to negativity Thus, after two years observation, subsequent to treatment, 19 (37 2 per cent) of the patients who received penicillin alone and 7 (14 3 per cent) who received combined therapy and negative spinal fluid examinations Only patients who had positive fluid examinations prior to treatment were included in these figures There is no doubt that the severity of the disease and the degree of deterioration, associated with the more highly positive spinal fluid formulae, account for much of these differences in the response of the spinal fluid abnormalities

With few exceptions, clinical remission has been accompanied by coincident improvement of the spinal fluid abnormalities In Figure 1, improvement in the several components of the spinal fluid is shown The cell count first reverted to normal, followed by a fall in the total protein Later, there was a reduction in the colloidal gold curve* and in the number of Kahn units The curves are so closely parallel that we are unable to determine any significant difference in the response of the various abnormalities to either method of treatment except that there is more rapid improvement after malaria plus penicillin due to the higher average of the spinal fluid components

At the end of two years the curves approximate each other

* These figures are the average of the total of the first three figures in the colloidal gold curve.

The difference in degree of activity between the two groups prior to treatment is offset by the fact that the curve for the combined group remains above that for the penicillin alone group in the instances in which an abnormality continues to exist

All that has been said about the two groups as a whole continued to be true when the various types of neurosyphilis were studied individually

RESULTS—ASYMPTOMATIC NEUROSYPHILIS

Twenty six patients with asymptomatic neurosyphilis were followed for a minimum of one year and eight were observed for two years after treatment (Table IV) Nineteen of these patients received penicillin alone and seven were treated with malaria and penicillin Six patients in the former and two in the latter group have been observed for at least two years

Since the patients had neither symptoms nor abnormal physical findings a clinical appraisal was not possible No patient in this group had developed symptoms, abnormal signs or progressive abnormalities in the spinal fluid since the completion of treatment At the end of one year 17 (89 per cent) of the 19 patients who received penicillin alone showed improvement in the spinal fluid abnormalities while in two instances the findings were unchanged After the same time interval six (86 per cent) of the seven patients who received combined treatment showed

TABLE IV

Results of Treatment of 34 Patients With Asymptomatic Neurosyphilis With Penicillin and Penicillin Plus Malaria

Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
		One Year Group—26						
Clinical	Penicillin	0	0	19	100	0	0	19
	Penicillin and Malaria	0	0	7	100	0	0	7
Spinal fluid	Penicillin	17	89	2	11	0	0	19
	Penicillin and Malaria	6	86	1	14	0	0	7
		Two Year Group—8						
Clinical	Penicillin	0	0	6	100	0	0	6
	Penicillin and Malaria	0	0	2	100	0	0	2
Spinal fluid	Penicillin	6	100	0	0	0	0	6
	Penicillin and Malaria	2	100	0	0	0	0	2

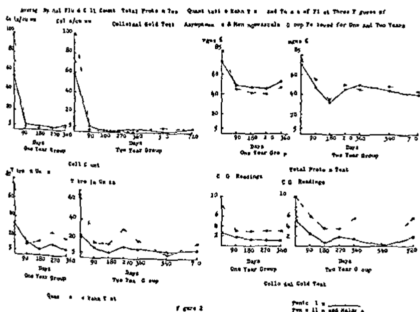


Figure 2

improvement in the spinal fluid findings, and one (14 per cent) was unchanged However, all eight patients (100 per cent), observed for two years subsequent to therapy, showed improvement in the spinal fluid abnormalities Six of these received penicillin alone and two malaria plus penicillin The response of the various components of the spinal fluid is shown in Figure 2 (The asymptomatic and meningovascular groups have been combined in Figure 2 for this study since they are relatively small groups usually represent eariler manifestations of the disease and respond in essentially the same manner to therapy)

At the end of one year the spinal fluid examinations of five patients (26 per cent), who received penicillin alone became negative Two of them who were observed for an additional year remained normal No patient in the malaria plus penicillin group reverted to negativity Perhaps this is explained by the higher average quantitative Kahn serological tests in the latter group (Figure 2)

Meningovascular Syphilis

There are 20 patients with meningovascular syphilis who have been observed for at least one year Twelve of these received penicillin alone and eight were given malaria plus penicillin Fifteen of these patients (nine who received penicillin alone and six who received the combined treatment) have been

TABLE V

Results of Treatment of 35 Patients With Meningovascular Neurosyphilis with Penicillin and Penicillin Plus Malaria

Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
		One Year Group—20						
Clinical	Penicillin	11	92	1	8	0	0	12
	Penicillin and Malaria	7	88	1	12	0	0	8
Spinal fluid	Penicillin	12	100	0	0	0	0	12
	Penicillin and Malaria	8	100	0	0	0	0	8
		Two Year Group—15						
Clinical	Penicillin	8	89	1	11	0	0	9
	Penicillin and Malaria	5	83	1	17	0	0	6
Spinal fluid	Penicillin	9	100	0	0	0	0	9
	Penicillin and Malaria	6	100	0	0	0	0	6

followed for a minimum of two years (Table V). Only one patient in each treatment group failed to respond clinically after one and two years of observation At the end of one year all patients (100 per cent) have shown improvement in the spinal fluid findings and this trend was maintained after a two year period of observation

Results—Tabes Dorsalis

This group is the second largest and consists of 63 patients who had tabes dorsalis Thirty-seven patients in this group received penicillin alone and 26 received both therapeutic malaria and penicillin Twenty-five of these 63 patients have been observed for a minimum of two years subsequent to treatment (Table VI) At the end of one year 25 patients (69 per cent) treated with penicillin alone had shown symptomatic improvement, 11 (29 per cent) were unchanged and one patient (2 per cent) was worse Eighteen (70 per cent) of the group that received malaria plus penicillin were improved at the end of one year, while eight (30 per cent) were unchanged and none were worse At the end of two years observation 15 of 19 patients (79 per cent) treated with penicillin alone were improved while four (21 per cent) remained unchanged Four (66 per cent) of the six patients who received malaria plus penicillin and were observed

TABLE VI

Results of Treatment of 88 Patients With Tabes Dorsalis With Penicillin and Penicillin Plus Malaria

Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
		One Year Group—63						
Clinical	Penicillin	25	69	11	29	1	2	37
	Penicillin and Malaria	18	70	8	30	0	0	26
Spinal fluid	Penicillin	30	81	7*	19	0	0	37
	Penicillin and Malaria	20	80	6**	20	0	0	26

* Includes 6 and ** includes 1 patients with negative spinal fluids before treatment

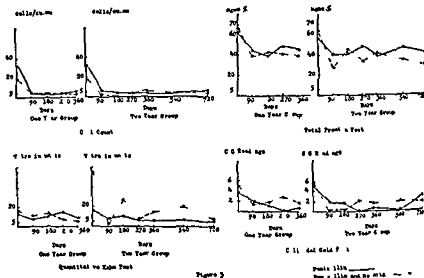
Appraisal	Treatment	Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
		Two Year Group—25						
Clinical	Penicillin	15	79	4	21	0	0	19
	Penicillin and Malaria	4	66	1	17	1	17	6
Spinal fluid	Penicillin	17	89	2*	11	0	0	19
	Penicillin and Malaria	6	100	0	0	0	0	6

* Both patients had negative spinal fluid before treatment

for two years after treatment were improved One (17 per cent) remained unchanged and one (17 per cent) was clinically worse

In no case did the spinal fluid formula become worse At the end of two years there was improvement of the spinal fluid findings in all cases that were abnormal before treatment The changes in the various cerebrospinal fluid tests are shown in Figure 3 Seven (23 3 per cent) of the patients with tabes dorsalis who received penicillin alone developed negative spinal fluid examinations at the end of one year In the group that received combined therapy five (26 3 per cent) reverted to negativity during the first year At the end of two years of observation eight (53 3 per cent) of the penicillin group had negative spinal fluids Four of these had become negative during the second year In the combined group two patients (40 per cent) had negative spinal fluid examinations two years after treatment one of these became negative during the second year

Three patients had tabes dorsalis associated with Charcot arthropathy Two patients developed the arthropathy subsequent to penicillin therapy and as would be expected none has improved



Seven patients with "burned out" tabes dorsalis had negative spinal fluid examinations, but suffered from repeated attacks of pain Five patients received penicillin only and two received malaria and penicillin There was improvement in the five patients who received penicillin alone One patient had gastric crises and improved during treatment, but has not been seen since treatment was completed With the exception of one patient who has shown gradual improvement over a period of one year, the patients have improved during the first three to six months after therapy and have remained essentially unchanged since then One of the two patients who received combined therapy was an opiate addict It is impossible to evaluate his clinical response The other patient treated with malaria and penicillin has shown no change

Results—Paresis

The largest group of this series consists of 71 patients with paresis and 43 patients with taboparesis They have been followed for one year Of this total of 114 patients, 56 have been followed for two years (Table VII) Forty of these patients received penicillin alone and 74 were given malaria and penicillin. Twenty-one of the former and 35 of the latter group have been observed for at least two years after treatment

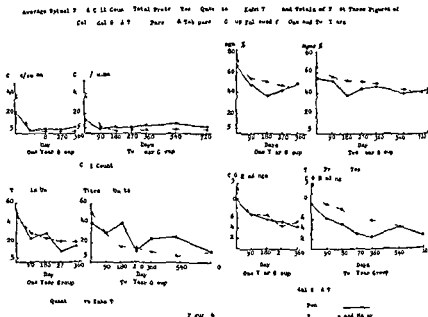
TABLE VII

Results of Treatment of 170 Patients with Paresis Including Taboparesis with Penicillin and Penicillin Plus Malaria

pra sal	Treatment	One Year Group—114 Improved No	Improved %	Unchanged No	Unchanged %	Worse No	Worse %	Total Number
cal	Penicillin	27	68	10	25	3	7	40
	Penicillin and Malaria	51	69	15	20	8	11	74
al flu d	Penicillin	34	85	6	15	0	0	40
	Penicillin and Malaria	65	88	7	9	2	2	74
		Two Year Group—56						
cal	Penicillin	13	62	6	29	2	9	21
	Penicillin and Malaria	23	66	8	23	4	11	35
al flu d	Penicillin	17	81	4	19	0	0	21
	Penicillin and Malaria	34	97	1	3	0	0	35

The penicillin alone group showed 68 per cent clinical and
per cent improvement in the spinal fluid findings in one year.
is is in contrast to 57 per cent and 70 per cent improvement,
pectively, in the previously reported smaller group[1] Those
o received penicillin plus malaria attained 69 per cent clinical
d 88 per cent improvement in the spinal fluid abnormalities as
npared to 64 per cent and 71 per cent, respectively, in the
lier report[1] No superiority of either method of treatment
demonstrated by the present figures At the end of a two year
st-treatment period of observation the penicillin alone group
owed 62 per cent clinical and 81 per cent improvement in the
nal fluid findings Those who received penicillin plus malaria
owed 66 per cent clinical and 97 per cent improvement in the
nal fluid examinations in two years

Seven (18 5 per cent) patients who received penicillin alone
d negative spinal fluid examinations by the end of the first
ar During the second year three (15 5 per cent) of 19 became
gative In the group treated with combined therapy, eight
1 8 per cent) spinal fluid examinations became negative dur-
g the first year and two of 31 (6 4 per cent) reverted to nega-
e during the second year of observation Thus, at the end of
two year post-treatment follow-up period seven patients (36 8
r cent) who received only penicillin and six (19 4 per cent)



who were given malaria plus penicillin had reverted to negativity

Figure 4 illustrates the response of the various cerebrospinal fluid abnormalities to the two methods of treatment The curves closely parallel each other, as in other types of neurosyphilis and there is no significant difference in the response except that again malaria plus penicillin produces improvement more rapidly

In the previous paper [1] malaria plus penicillin was considered to be the treatment of choice for paresis but with a larger group of patients and a longer follow up period its superiority was less evident The combined therapy showed a 16 per cent superiority over penicillin alone in producing improvement in the spinal fluid abnormalities after two years of observation After one year of observation this superiority was only 3 per cent higher with the combined therapy than with penicillin alone These figures indicate that patients must be observed for a longer period before definite conclusions can be reached The other comparative studies indicate little if any added improvement when fever therapy is combined with penicillin Clinically there was no significant difference in the two groups after one and two years

COMMENT

Progressive improvement in the post treatment cerebrospinal fluid is the best indicator of the adequacy of treatment in cases of neurosyphilis, and the demonstration of an arrest and resolution of the disease is based, in the main, on the attainment of a normal spinal fluid "Improvement' and 'progression" are difficult to define in cases of central nervous system syphilis because physicians vary in their interpretations of the clinical phenomena involved the reaction to the disease is different in each patient and in the same patient, from visit to visit Because of this the cerebrospinal fluid abnormalities comprise the most substantial basis for analysis of a group of patients with neurosyphilis The clinical result is relegated to second place because of the frequent inability to accurately evaluate the often slow, varied and insidious "improvement" or "progression" of symptoms

The group of patients with asymptomatic neurosyphilis permit the most clearly defined opportunity for a comparison of the effect of the two forms of therapy on the spinal fluid abnormalities These cases require no discussion It is evident from the results that there is no value in the addition of therapeutic malaria to a course of 4 000,000 units of penicillin At the end of two years observation, subsequent to treatment, there was improvement in all cases and in no patient has there been a relapse Penicillin alone is the treatment of choice in asymptomatic neurosyphilis The same can be said in cases of meningovascular syphilis and tabes dorsalis

Taking into consideration morbidity and mortality as well as therapeutic effectiveness penicillin alone is far less dangerous and probably equal in therapeutic effectiveness to the combination of penicillin and malaria in the treatment of general paresis There may possibly remain some evidence that fever therapy plus penicillin is superior to penicillin alone in dementia paralytica We have the impression that a longer period of observation after treatment will show improvement from penicillin alone equal to that from combined therapy

One hundred and nineteen (53 4 per cent) of our patients had at least minimal adequate antiluetic therapy prior to being examined by us Almost without exception this was not given during the early stages of the disease Ninety-seven (81 per cent) of these patients later showed improvement following treatment of their neurosyphilis

Age and sex of the patient do not seem to contribute to the number of failures The chief cause of progression of the disease is in the individual's inability to react favorably to penicillin or fever therapy Neither penicillin nor therapeutic malaria can erase degeneration or replace scar tissue and these appear to be the basic causes for failure even though treatment has been adequate If patients with neurosyphilis can be treated with an adequate dosage of penicillin before symptoms, the majority will suffer no disability from the disease

We have not been able to correlate changes in the blood serological tests with clinical improvement or lowering of the spinal fluid formulae The majority of patients with positive serological tests in the serum have maintained some degree of positivity in spite of clinical improvement and/or improvement in the spinal fluid abnormalities

Summary

1 From a total of 539 patients with neurosyphilis who have been treated with penicillin alone or penicillin and malaria 223 were observed for one year and 104 for two years after treatment

2 Patients with asymptomatic neurosyphilis meningovascular syphilis and tabes dorsalis respond as well to a penicillin alone regimen as one of penicillin and malaria This response was apparent in both the clinical evaluation and spinal fluid improvement of these patients

3 Patients with paresis and taboparesis responded as well in the clinical evaluation of their symptoms to penicillin alone as they did to a combination of penicillin and malaria The latter regimen retains a slight superiority after two years of observation if the improvement in the spinal fluid is used as the sole index of comparison A longer period of post treatment observation seems indicated before any final conclusion can be drawn regarding the efficacy of penicillin alone in the treatment of this group

4 The response of patients with the several types of neurosyphilis seems to be more rapid when the combination of penicillin and malaria is used Even in taboparesis and paresis there are indications that penicillin therapy alone will eventually prove as effective as its combination with malaria Penicillin therapy alone is certainly far less hazardous

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DISCUSSION OF PAPERS ON NEUROSYPHILIS

Evan W. Thomas (Bellevue Hospital): I think it important that a more uniform terminology be adopted to describe the spinal fluid findings of patients with neurosyphilis The terms "near normal" and "practically negative" are vague and subject to misinterpretation The term "Dattner-Thomas spinal fluid formula" also has little meaning unless everyone understands what Dattner and I mean by "active" and "inactive" spinal fluid findings In most cases of active syphilitic inflammation of the central nervous system the spinal fluid will not only have positive specific tests for syphilis but also have increased cells and protein and abnormal colloidal tests The cells and increased protein are evidence of an active inflammatory process Following successful treatment of neurosyphilis healing of syphilitic lesions begins to occur The healing process is reflected in the spinal fluid findings with the result that cell counts become normal within 3 months after treatment and abnormally high total proteins begin to fall towards normal levels Quantitative complement fixation tests of the spinal fluid and abnormal colloidal tests also show trends toward normal values, although they usually remain abnormal for longer periods than the other two tests Following therapy, as long as the cell count is normal and there is a satisfactory drop in the total protein, Dattner and I use the term "inactive spinal fluid" to indicate that the syphilitic process in the central nervous system is no longer progressing but is healing We do not believe that it is necessary or advisable to give further antisyphilitic treatment to patients whose spinal fluid findings indicate an arrested rather than a progressive syphilitic inflammation

As a brief descriptive term is desirable to differentiate between spinal fluid findings which indicate syphilitic activity and inactivity, we suggest the terms "active spinal fluid" and "inactive spinal fluid" rather than such phrases as "near normal" or "practically negative"

PENICILLIN IN TREATMENT OF PRIMARY OPTIC ATROPHY

By
*Joseph V Klauder**

As of April 1 1948 forty nine patients with primary optic atrophy as part of neurosyphilis have been treated with penicillin.

Of the forty nine patients 13 were white and 36 were Negroes Of the white patients 9 were males and 4 were females of the Negro patients 27 were males and 9 were females

Forty six of the forty nine patients had acquired syphilis the remaining three were females who had congenital syphilis their ages were 15 21 and 28 years respectively The age range of the forty six patients with acquired syphilis was as follows

27 30	31 35	36-40	41 46	47 55	56 66
3	6	4	15	12	6

Of the patients with acquired syphilis 28 h d tabes 17 dif fuse meningovascular neurosyphilis and 1 had gen^r^l paralysis

When penicillin was first employed in treatment in April 1944 commercial sodium penicillin was employed injections were given every three hours for seven and one half days for a total dose of 4 200 000 units No other treatment was given unless the optic atrophy unfavorably progressed retreatment with peni cill n was then instituted as well as fever and chemotherapy Thirty one of the forty nine patients were treated according to th s schedule

About August 1946 a new schedule of treatment was institut ed This consisted of 3 6 grams of crystalline Penicillin G (the equivalent of about six million units of commercial sodium peni cill n) Injections were given every three hours for seven and one half days Fever therapy (eight to twelve fever rises) was given conjointly with penicillin Malaria was favored if the phy ical condition of the patient permitted quartan malaria being employed for the Negro patients No subsequent treatment was given unless the optic atrophy unfavorably progressed Seven teen patients were treated with this new schedule

The status of the optic atrophy of the forty nine patients is shown in Table I

As shown in Table I the opt c atrophy of 42 of the 49 pa t ents was progressive at the time penicillin was given Of these

* W lls Hosp tal Ph ladelph a

TABLE I

COURSE OF OPTIC ATROPHY AFTER PENICILLIN—49 Patients

Twenty Patients Had Treatment before Penicillin, Twenty nine Did Not

Before Penicillin Therapy		After Penicillin Therapy							
Progressive (42 cases)	No Change (27 cases)	Mos Observ	*48-44*	*43 37*	*36-28*	*22-18*	*17-12*	*11-7*	*1-2*
		No of Cases	1 2[a]	4[a]	2,2[a]	4,1[a]	2,1[a]	3,1[a b]	4[a]
	Worse (9 cases)	Mos Observ	*48-44*		*36-35*	*26*	*12*	*3*	
		No of Cases	1[a],2[a c]		1,1[a],1[a c]	1[c]	1[c]	1[a]	
	Blind (6 cases)	Blindness in months after penicillin	*24*	*18*	*12-8*	*3*			
		No of Cases	1[c]	1[a c]	2[a],1	1[a]			
Stationary (7 cases)	No Change (7 cases)	Mos Observ	*48-42*	*31*	*25*	*2*			
		No of Cases	3,1[a]	1	1	1[a]			
	Worse		None						
	Blind		None						

a—Visual acuity 6/60 or less in both eyes at time of penicillin therapy
b—1 deceased
c—Retreated with penicillin

36 patients optic atrophy remained stationary (no unfavorable progress) in 27 The period of observation after penicillin treatment of the twenty seven patients ranged from a few months (5 recently treated patients) to a maximum of 48 months Optic atrophy became worse (unfavorable progress) in 9 The period of observation after penicillin treatment of these 9 patients ranged from 1 to 4 years for 8 patients and 3 months for 1 patient Of the 9 patients, 5 were industrially blind at onset of penicillin treatment and 6 were retreated with penicillin at first evidence of unfavorable progress Six patients became blind, 3 of these patients however, were industrially blind at time of penicillin treatment and 4 were retreated Despite retreatment blindness ensued Blindness occurred in from 8 to 24 months after the first penicillin treatment

The optic atrophy of 7 of the 49 patients was stationary at time of penicillin treatment The disease remained stationary in all of the 7 patients The periods of observation ranged from 25 to 48 months except for 1 patient it was 1 month

Of the 49 patients treated with penicillin, 27 patients were industrially blind (visual acuity of 6/60 or less in both eyes) when first seen and at time of penicillin treatment Of these 27 patients the optic atrophy remained stationary in 17 for a period of observation ranging from a few months to 48 months, six patients became worse and four became blind, blindness ensued in 8 to 18 months after penicillin

Of the 49 penicillin treated patients, 20 were treated before instituting penicillin therapy Of these 20 treated patients, four had fever therapy, 3 induced by malaria and 1 by vaccines intravenously, and two had Swift Ellis treatment In addition chemotherapy was given and was also used in the remaining number of the treated group Of the 20 treated patients the progress of their optic atrophy after penicillin treatment was as follows No change in 14 became worse in 5, and became blind in 1

Of the 49 patients 25 had no treatment prior to penicillin Of these 25 patients the progress of their optic atrophy after penicillin was as follows No change in 14, became worse in 5 and became blind in 5

The aforegoing results of penicillin therapy I regard as encouraging I would hesitate to draw conclusions until penicillin treated patients are observed for a longer period and comparison is made with non penicillin treated patients

For purpose of this comparison, 400 case records of patients

quent to treatment, penicillin alone is equal in effectiveness to therapeutic malaria

5 Penicillin is not the ideal drug for the treatment of syphilitic primary optic atrophy and, like fever therapy, fails to stop the process in a certain number of patients The decision as to whether penicillin will be an adequate substitute for therapeutic malaria in this disease awaits a longer period of post treatment observation of a larger group of patients If it does prove to be as effective as fever therapy, it will offer a form of therapy that is much less hazardous to the patient

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SIXTY CASES OF PRIMARY OPTIC ATROPHY CRITICALLY STUDIED FROM THE STANDPOINT OF DIAGNOSIS AND THERAPY*

By

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with the collaboration of John H Stokes, Herman Beerman Norman R Ingraham, Jr, Mortimer S Falk, George D Gammon Paul Gyorgy, Elizabeth K Rose, and John W Lentz and the technical assistance of Verna Mayer Stein Emily Stannard Jane Barbara Taylor, and Mildred F Wills **

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* This investigation also was assisted by contributions from the Gar Wood Fund and the Kirby McCarthy Fund

** From the Institute for the Study of Venereal Disease University of ... ealth Service cooperating and ... rsity Hospital The Institute ... ogy, Dermatology and Syphilol ... nereal Disease Control Phila ... and the Philadelphia General and Pennsylvania Hospitals and the Children s Hospital are represented in the authorship

The patients reported upon here are from the Penicillin-Syphilis group at the University of Pennsylvania All are neurosyphilitics, all have received penicillin systemically as the major therapy, although many of course had various grades of therapy prior to penicillin. These patients have been studied from the standpoint of primary optic atrophy and the results of penicillin therapy The clear-cut and resounding title of the report should be modified in several ways as we shall presently see, and should convey some suggestion of the difficulties encountered in an analysis of this nature The title, by the way, is Dr Stokes, not mine It might also be added that the reluctance on the part of the ophthalmology side of the Panel to speak out at all at this time has been overcome by the very persuasiveness of Dr Stokes, who feels, I believe, that after four years of silence this aspect of the study should be given voice if only to prove its existence That very little else is proven will shortly become apparent.

Of the 537 cases of all types of neurosyphilis studied during a four year period, 100 cases were given a working diagnosis of primary optic atrophy following visual acuity, visual field, and ophthalmoscopic examinations (Table 1) What has happened to these 100 cases?

After repeated follow up examination and evaluation 29 of the original 100 cases have been shown definitely not to have syphilitic primary optic atrophy, and 23 are still carried as uncertain but probably not POA, a total of 52 thus being excluded, leaving only 48 cases in which the diagnosis is without question

The findings in the 29 cases definitely excluded have been explained by a variety of conditions (Table 2)

TABLE 1

GENERAL SURVEY OF PATIENTS

Total number of neurosyphilis patients		537
Of which diagnosis of POA made on		100
Of these we excluded from study		52
Because		
Definitely not POA	29	
Probably not POA	23	
Leaving as definite cases of POA only		48

year of treatment, the other five having remained the same for 2-4 years, four of which however having been in the same stable category several years prior to penicillin

We are thus reduced to 31 cases of POA who had enough remaining useful vision at the start to permit therapeutic evaluation It is manifestly fruitless to attempt a statistical study of 31 cases followed for from 6 months to 4 years, so that comparison with other forms of therapy or with other published series is impossible A statement of facts in connection with these cases will hence be concluded by some very limited impressions

Table 6 shows the duration of follow-up post-penicillin of the 10 cases considered worse and the 21 cases considered stable

TABLE 6

Duration of Follow Up Post Penicillin of 31 Cases of POA

Months	Stable	Worse	Total
6-12	2	0	2
12 24	7	3	10
24-36	8	4	12
36-48	4	3	7
Totals	21	10	31

There is no great discrepancy evident between the two categories as compared with the total, although it might be agreed that the cases considered worse are weighted slightly on the long follow up side Yet an almost equal portion of the stable cases is on the long end of the follow-up This is one of the many questions requiring further time

Table 7 shows an analysis of the 10 cases which have become worse following penicillin therapy

The first striking fact is that 8 of the 10 had vision less than 10/200 in the one eye before treatment, one other having vision in the bad eye reduced to 20/200 Only one case (876) had findings limited to disc pallor and field changes Thus the majority of the cases which have grown worse were moderately advanced prior to penicillin The first six cases are worse by further acuity loss, the last four having maintained their acuity but lost field Only two eyes were completely blind before, six

TABLE 7

Ten Cases Worse Post Penicillin

Case No	Vision Before		Months Since Penicillin	Vision After	
	OD	OS		OD	OS
391	20/70	L.P	35	L.P	Blind
979	3/200	20/40	17	3/200	C.F
60	20/200	20/[illegible]0	31	Blind	20/[illegible]0
18	20/70	Blind	34	3/200	Blind
6[illegible]0	L.P	20/50	12	Blind	15/200
3	20/200	3/200	47	6/200	3/200
876	20/15	20/15	22	20/15	20/15
212	H M	20/200	42	H M	20/200
1[illegible]0	H M	20/100	42	Blind	20/100
469	[illegible]0/[illegible]0	Blind	28	20/30	Blind

after Although 8 eyes were Moore-Woods blind before, 13 were after Four cases are now Moore-Woods blind in both eyes Yet 3 cases well advanced at the start have not lost further central vision

How do these observations contrast with the stable group? (Table 8)

Twelve out of 21 stable cases were Moore-Woods blind pre-penicillin compared with 8 out of 10 worse cases Seven of the 21 stable cases have never had symptoms and are diagnosed on the basis of disc and field changes only, while only one of the 10 worse cases is so classified There appears to be no difference in the average duration of symptoms where present between the two groups, and the proportion of patients considered to have

TABLE 8

Comparison of 21 Stable and 10 Worse Cases

	Stable	Worse
Pre-penicillin vision one eye less than 10/200	12	[illegible]
Symptom free	7	[illegible]
Inadequate previous therapy	12	[illegible]
Duration of symptoms where present (average)	6 yrs	[illegible]

after penicillin administration Three of these patients who were not in cardiac failure at the time of treatment are still compensated and are receiving digitalis One patient who was not decompensated at the time of treatment went into cardiac failure four months afterwards and is on a rigorous cardiac regime at the present time The fifth patient, in cardiac failure prior to treatment, has since required hospitalization due to limited cardiac reserve

Among the twenty five cases of aortic regurgitation, five have died and twenty survive Of the five deaths four certainly and one probably died a cardiac death These deaths occurred from between 21 and 932 days after the administration of penicillin One in cardiac failure before penicillin survived 50 days, two others decompensated shortly before the time of death

The twenty survivors have been followed for from 158 to 1243 days (mean 567 days) Of these fifteen never in cardiac failure before penicillin are still not decompensated Only two of these are receiving digitalis Of the five who were decompensated prior to penicillin two are still in failure (invalids) on good medical cardiac regimes The other three are not now decompensated but one is blind due to syphilitic primary optic atrophy, and the other is permanently hospitalized due to a cerebral vascular accident Had these last patients not had these concurrent restricting conditions they might well have become cardiac invalids All are receiving digitalis and the usual cardiac management

No conclusions can be drawn at this time concerning the eventual outcome of patients with cardiovascular syphilis to whom penicillin has been administered The periods of observation after treatment are all less than four years The number of patients with aneurysms is too small to be of statistical value Of the patients with aortic regurgitation who were in cardiac failure prior to treatment no significant improvement in cardiac status was observed as a result of the administration of penicillin It would appear as though the natural course of the disease at least for the first four years is not greatly affected by the administration of penicillin

TREATMENT OF INFANTILE CONGENITAL SYPHILIS

RESULTS OF AQUEOUS PENICILLIN ALONE IN 60 INFANTS FOLLOWED FOR AN AVERAGE OF TWO YEARS AFTER TREATMENT*

By

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with the collaboration of John H Stokes, John W. Lentz, Virgene S Wammock, O M Carrozzino, and Herman Beerman, and the technical assistance of Emily Stannard, Verna Mayer Stein, Jane Barbara Taylor, and Mildred F. Wills **

In the treatment of the infant with congenital syphilis, general experience to date has proved penicillin the most satisfactory therapeutic agent Long term follow up reports, however, have been lacking and it is for this reason, therefore, that our study based on 60 infants† followed over an average of two years is presented Previously, seemingly adequate antiluetic therapy in infancy has been followed by late relapses, the development of juvenile paresis or interstitial keratitis

A previous report[1] discusses selection of cases, dosages, reactions, complications, deaths, and immediate response to

* The work described in this paper was done under a grant from the Research Grants Division of the National Institute of Health in continuation of a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of P

** ... of Venereal Disease University of ... blic Health Service cooperating and ... University Hospital The Institute ... philol ... hila ... neral ... d in

† ... Hospital supplied ... those infants the ... pital of the ... Luke's and ... Hospital

TABLE I

INTRAMUSCULAR PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Age at Onset of Treatment and Mortality

Age	Total Number Treated	Number Dead	Per Cent Mortality
Under 3 months	33	6	18.2
From 3 to 6 months	10	1	10.0
From 6 to 12 months	5	0	0
From 12 to 24 months	5	0	0
Over 24 months	2	0	0
TOTAL	55	7	12.7

treatment This report concerns itself more with long term observation to see whether or not there may occur clinical or serological relapses in spite of initial clinical improvement—or whether or not any other complications or sequelae of congenital syphilis may occur

Table I shows an analysis of the age at onset of therapy in the 55 patients treated with intramuscular penicillin alone Five other patients receiving penicillin by mouth will be mentioned subsequently No previous antiluetic treatment had been given except in 2 of the 60 children In both instances it was considered too inadequate to have influenced the results in our study No patient was accepted for treatment without a positive serologic test for syphilis and in addition either dermatologic or roentgenographic evidence of the disease Aqueous penicillin of known brand and lot number was given intramuscularly every 3 hours in periods varying from 7 to 16 days Dosage varied as experience grew During the earlier part of the study about 11 000 20 000 units per pound (5000 9090 units per kilo) of body weight for the total dose was employed This was gradually increased to 200 000 units per pound (90 909 units per kilo) of body weight given over a period of 15 days in equal doses every 3 hours

In none of the seven deaths discussed fully in our previous report was it felt that the penicillin therapy was related to the outcome Two died later at home of other causes after marked clinical improvement in their syphilis The other five were all very small debilitated infants who probably never would have lived to have been included in the study had supportive treatment without penicillin been inaugurated first In every case treatment was begun immedaitely as soon as the diagnostic criteria were satisfied

TABLE II

INTRAMUSCULAR PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Various Dosages Used

Number of Patients	Dosage—Units per Pound Body Weight	Per Kilo
29	Up to 40 000	Less than 18 000
14	40 000 80 000	18 000 36 000
11	80 000 200 000	36 000 90 000
1	Over 200 000	More than 90 000
55		

Table II shows the number of patients on the various dosages Follow up on our treated patients included regular visits at which time were noted serologic titers physical examination and roentgen examination of the bones if previous abnormalities existed Yearly examination of cerebrospinal fluid was planned The average length of the period of observation is two years on 52 of the 53 surviving patients —only one patient having been lost to follow up The shortest follow up periods were 59 85 99 and 106 days the longest 1339 1343 1362 and 1432 days Table III shows the duration of observation Our plan is to continue yearly examinations of these children with especial attention to their serologic titers growth spinal fluid and possible complications such as interstitial keratitis or juvenile paresis in the seroresistant cases

TABLE III

PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Duration of Observation

Number of Patients	Period of Observation
12	Under 1 year
19	Between 1 and 2 years
11	Between 2 and 3 years
10	Over 3 years
1	Lost to Follow up
7	Died
TOTAL 60	

Response to therapy is shown in Table IV with relation to the age at onset of treatment In this group of [illegible] 3 months of age there were 33 infants of whom 27 and have been followed All are clinically well remained seronegative One infant still under [illegible] only 85 days since onset of treatment is still [illegible] the titer is declining None has relapsed Sero [illegible]

TABLE IV

INTRAMUSCULAR PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Outcome Analyzed as to Age at Which Treatment was Started in 55 Patients Followed an Average of Two Years

Age at Onset of Therapy	Total Number Treated	Number Dead	Number Living	Number Apparently Cured (Seroneg)	Clinically well, seropositive or declining titer	No Change
Under 3 mo	33	6	27	27	0	0
3-6 mo	10	1	9	5*	3	1 lost
6-12 mo	5	0	5	2	3	0
12 24 mo	5	0	5	3	0	2
Over 24 mo	2	0	2	0	1	1
TOTAL	55	7	48	37	7	4

* Retreated at 177 days Darkfield positive Seronegative 289–1432 days

formly fell and on an average of all patients were seronegative and remained so from 198 days on This figure might have been less had regular monthly titers been obtainable on all patients, but this difficulty in regular follow-up is common to all such studies The rate of the fall in titer seemed to bear no relation to the amount of penicillin used, as the total dosages in this group varied from 11,000 to 180,000 units per pound (5,000 to 81,800 units per kilo) of body weight

In the next older group of 9 infants (between 3 and 6 months of age at onset of treatment) 3 have become and remained seronegative at 135, 350, and 365 days Another was seropositive at 83 days but when next seen at 689 days had become seronegative Another, Case No 58, treated at 16 weeks with 18,000 units per pound (8,000 per kilo) was re treated at 177 days when he showed a positive darkfield relapse, became seronegative at 289 days and has remained consistently so through 1432 days Three others are clinically well, yet two are still seropositive at 85 days and the other doubtful at 414 days One was lost to follow-up Here again the clinical response seemed unrelated to the amount of penicillin used,—the dosages ranging from 18,000 to 180,000 units per pound (8,000-81,000 per kilo) of body weight

In the 5 infants between 6 and 12 months of age all responded well clinically, but 3 are still showing declining titers—4 Kline units at 106 and 208 days respectively in 2, the third showing a doubtful reaction at 612 days Interestingly enough this last patient, S M No 900, received the largest dose of any of our

TABLE V

INTRAMUSCULAR PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Clinically Well, Still Seropositive

Case No.	Age at Onset of Treatment	Dosage of Penicillin Units per Pound Body Weight	Comment
1198	3½ mo	180 000 (80 000 units per kilo)	Still 256 Kline units at 85 days
868	5 mo	70 000	Declining doubtful at 464 days
1263	5½ mo	180 000	[illegible]
500	6 mo	455,184	[illegible]
1171	7 mo	180 000	[illegible]
63	9 mo	20 000	[illegible]
965	16 mo	40 000	[illegible] ine
1266	18 mo	200 000	[illegible] ine
378	30 mo	20 000	[illegible] ys 474 days
835	2 yrs	70 000	Declining, 64 Kline units at 538 days

patients, 455,000 units per pound (207,000 per kilo). This dosage was chosen to see what, if any, effect it might have on the marked and extensive osseous lesions. This phase of our study will be discussed in a subsequent report.

In the 5 infants between 12 and 24 months of age all are clinically well, but vary in serologic responses. Only 2 are seronegative—one at 314 days and another at 955 days, but only after being re-treated at 386 days. Another showed a declining titer at 59 days, when he was lost to follow-up. Another shows no change in titer yet received his treatment of 200,000 units per pound (90,000 units per kilo) of body weight only 92 days ago. Another infant, after initially receiving 40,000 units per pound (18,000 units per kilo) and still showing 256 Kline serologic units, is now being re-treated at 520 days. Of the 2 infants over 2 years of age, both are well, but still seropositive. One showed a declining titer 538 days after having received 68,000 units of penicillin per pound (30,900 units per kilo) of body weight. The other was re-treated 215 days after an initial dosage of 20,000 units per pound (9,000 units per kilo) because her serum still showed 128 Kline units. She has since moved to

TABLE VI

PENICILLIN ALONE IN INFANTILE CONGENITAL SYPHILIS

Penicillin By Mouth—Five Cases

Case No	Age at Onset of Therapy	Dosage in Million Units	Comment
473	20 wks	1 5	St 1:256 at 385 days when retreated with 1 2 mil neg at 821 days
474	18 wks	1 5	
475	7½ wks	1 3	
509	12 wks	2 5	
592	2= yrs	2 25	

North Carolina where a positive serologic test was reported at 474 days

In the 16 patients in whom repeated examinations of the cerebrospinal fluid have been obtained, no abnormalities have been noted These patients all had satisfactory serologic and clinical responses

An attempt was made to study the effects of penicillin given by mouth Five infants were treated A buffered preparation of calcium penicillin was used in dosages varying from 1 5 to 2 5 million units Tablets of 12,500 units were given every 3 hours No untoward effects were noted except loose stools occasionally in the younger infants Results are noted in Table VI Here again it is noted that the best results were obtained in the younger infants

Discussion

It would seem then that one might expect good clinical response to congenital infantile syphilis treated with intramuscular penicillin within a fairly wide dosage range Perhaps the most important factor favoring good response is the age of the patient at the onset of treatment —the younger the patient the better the outcome The fact that there was not a single treatment failure in the surviving infants, treated under the age of three months is particularly noteworthy This is in accord with observations in the experimental animals and in the adult,—that syphilis is very amenable to treatment in its initial stages Although clinical improvement is an almost uniform response in all ages, yet the fall in serologic titer becomes increasingly less prompt and persistent in the older infants Penicillin was thought to have no relationship to the cause of death in our infants This has been previously reported[1]

The long term follow-up study here presented seems to bear out the previous impression showing the remarkable superiority of penicillin treatment in congenital infantile syphilis over all other previous therapeutic schemes, and recommendations,—not only with regard to the safety and relatively short duration of therapy but also in respect to the lack of clinical and serologic relapses There is no indication that various penicillin preparations (amorphous or crystalline) behaved differently

SUMMARY

Sixty infants with congenital syphilis have been treated with penicillin and followed for periods up to three years

Of the 53 living patients, only 1 has been lost to follow-up

Of the 48 living patients treated with intramuscular penicillin 37 are apparently cured (clinically well and seronegative), 7 are clinically well but still seropositive, though titer declining, 3 show no change in titer. Two of the latter have recently been re treated

Five patients were treated with penicillin by mouth —4 infants responding satisfactorily, one older child of 2-1/3 years well, but seropositive after re-treatment

The age of the patient at the onset of treatment rather than the dosage, type or means of administration of penicillin, seemed to be the chief factor in determining satisfactory response, cures approaching 100 per cent if treatment is commenced before the third month of life The amounts of intramuscular penicillin varied from 20,000 to 200,000 units per pound (9 000-90,000 per kilo) of body weight Even in this relatively small group of 53 patients studied over prolonged periods up to three years, the absence of serologic relapse, except in the one case cited, should be emphasized

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THE NECESSITY FOR TREATMENT OF PREGNANT SYPHILITIC WOMEN DURING EVERY PREGNANCY*

By

Mary Stewart Goodwin, and *Mary Streeter Farber*, Baltimore

The prevention of prenatal syphilis by treatment of the infected mother during pregnancy is one of the major public health triumphs of medicine The results in terms of normal living infants, already fairly satisfactory with arsenic and bismuth metal chemotherapy, have been still further improved upon by penicillin, which is spectacularly and nearly completely successful in protection of the fetus[1]

All workers in this field have, however, been plagued by the question —is it necessary to treat a syphilitic woman during every pregnancy? The literature in this country and abroad contains the repeated recommendation that the syphilitic mother should be given "adequate" treatment** in every pregnancy, with only a few guarded suggestions that a less conservative policy might eventually be adopted

Cole and his co authors of the Cooperative Clinical Group[2] reported that in a group of 52 syphilitic women considered "cured" no syphilitic child resulted from subsequent pregnancies during which no treatment was given Nevertheless, they consider the syphilitic mother a "potential reservoir of infection for the fetus she carries even though she can no longer transmit the disease to others", and recommend unequivocally the repetition of treatment in each pregnancy Stokes[3] advocates the treatment of the syphilitic woman "through every pregnancy regardless of the duration of her infection, her serologic status or the amount and type of antecedent therapy", but adds the

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* From the Johns Hopkins University and United States Public Health Service Venereal Disease Research and Post Graduate Training Center

* This investigation was aided by grants from the Carolina Rose Strauss Foundation Monroe La the Maryland State Board of Health and the Research Grants and Fellowships Division National Institute of Health United States Public Health Service

** The word 'adequate applied to antisyphilitic therapy, is abused and overworked As employed in this paper it is intended to mean adequate for protection of the fetus

comment that some modification of this position may be possible when reports are available of an extensive series of mothers clinically "cured" of the disease who have gone through subsequent pregnancies untreated

As late as 1941, Moore[4] stipulated that retreatment of a previously treated mother may be safely omitted "if the mother's infection is of more than 10 years' duration; if previous treatment has been adequate to permit the probation of the mother, and especially if several uneventful and seronegative years have passed since the termination of treatment; and if one or more preceding pregnancies during which the mother has been allowed to go untreated, have resulted in living children known to be non-syphilitic." He felt that if at least two of these four criteria could not be satisfied, "it is safer to treat the mother during the pregnancy in question, in spite of the fact that her own blood tests may be repeatedly negative" He agreed with McKelvey and Turner[5] that thorough treatment of the maternal syphilis prior to pregnancy probably affords adequate protection of the offspring in subsequent pregnancies even though treatment during pregnancy is omitted, but advised that if the maternal serologic test remains positive, or if there is clinical evidence of persistent infection, the mother should be treated during every pregnancy regardless of the amount of previous treatment

A few small groups of syphilitic women untreated during a given pregnancy have been reported in the last 20 years Birnbaum[6] noted 21 such women, considered clinically "cured," who had 34 subsequent pregnancies without further treatment, and normal non syphilitic children in all cases Rattner[7] reported 5 such patients, and Findlay[8], on the basis of his personal but undocumented experience with "adequate" antisyphilitic treatment, felt that it was not necessary to follow the usual recommendation of retreatment through all pregnancies

Because of the individual and public health importance of the problem, a planned study was cautiously undertaken in 1939 in the Family Syphilis Clinic of the Johns Hopkins Hospital Selected syphilitic mothers, treated before or during a previous pregnancy, were deliberately allowed to go through one or more subsequent pregnancies without additional treatment and the infants born of these later pregnancies were carefully followed and studied to determine the presence or absence of syphilitic infection in them

METHODS AND MATERIALS

The original criteria for admission of a woman to the study were —(1) a reasonably accurate history or, if a definite history was lacking, a reasonable presumption (on the basis of collateral evidence) of maternal infection of at least two years' duration prior to the pregnancy in which she was to be deliberately untreated; (2) for which she had completed "adequate" treatment* prior to the pregnancy under observation, (3) negative maternal blood serologic tests for syphilis (subsequently abbreviated STS) before and during the current pregnancy, (4) normal maternal spinal fluid test; (5) no evidence of active maternal infection on physical examination; and (6) at least one previously successful pregnancy (not necessarily occurring *after* the previous treatment) When an appreciable number of women had been observed without an infantile failure, these criteria were relaxed to include some women whose infections were of less than 2 years' duration at the time of the pregnancy in which treatment was to be omitted, provided "adequate" treatment had been completed prior to the onset of this pregnancy, some whose blood serologic tests were still positive in spite of previous treatment, if physical evidence of active infection was lacking, some (98 in number) who did not have spinal fluid examinations, and a few (12 cases) with abnormal but inactive spinal fluids (in the Dattner-Thomas sense of normal cell count and protein content)

All mothers in the series had quantitative STS and physical examinations at monthly intervals throughout the pregnancies during which treatment was omitted This was considered to be essential in order to detect at once any evidence of reactivation of the original maternal infection or signs of reinfection That the latter is a real hazard is indicated by the frequency with which infectious syphilis is encountered late in pregnancy in our Baltimore clinic population If serologic or clinical relapse or reinfection was detected, the woman was promptly retreated and dropped from the study

All infants were followed with serial serologic and physical examinations, and where possible with serial roentgenograms,

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* In this group of patients, previous 'adequate' treatment was arbitrarily defined as 2 0 4 0 gm or more of arsphenamine, i e, 6 12 or more injections of this drug (or equivalent dosage with other arsenical drugs) plus concomitant bismuth or mercury, whether given before, during, or after a previous pregnancy See below for further discussion of this point

according to well established procedure for the diagnosis of infantile congenital syphilis[3].

The group available for analysis now contains 385 women, among whom the previous treatment administered was metal chemotherapy (arsenic and bismuth or mercury) in 363, penicillin in 22. These two forms of treatment will be considered separately

RESULTS AFTER PREVIOUS METAL CHEMOTHERAPY

This group of 363 mothers include 331 Negro and 32 white women. Their ages, at the time of the original diagnosis of syphilis in themselves, varied from 3 months to 40 years The majority (272) were first recognized as syphilitic between the ages of 15 and 30 years

The types of maternal syphilis diagnosed at the time of original treatment are indicated in Table I, together with the duration of maternal infection at the time of the pregnancy which treatment was to be withheld Only 26 mothers had been infected for less than 2 years when the pregnancy in question occurred These were women admitted late in the study, who had early syphilis when first seen, promptly received "adequate" continuous therapy, became seronegative before the completion of this treatment, and remained both seronegative and clinically well throughout the observed pregnancy

In McKelvey and Turner's[4] earlier study from this clinic, it was demonstrated that of 59 mothers who received more than ... gm. (34 injections) of arsphenamine *prior* to but none during pregnancy, not one had a syphilitic baby The interval between treatment and the occurrence of pregnancy in these women ranged from a week to several years Their study also showed that of 19 women who received more than 4 grams of arsphenamine *during* a pregnancy, the offspring were all normal and non-syphilitic These results guided us in admitting women to our study

As indicated in Table II, 332 women had received more than ... gm. of arsphenamine (or arsenical equivalent) plus bismuth or mercury, prior to the onset of the observed pregnancy Eighteen had received 2-4 gm, with satisfactory clinical and serologic response to treatment and seronegativity sustained throughout the observed pregnancy Three women had a total of less than 2 gm of arsphenamine prior to treatment

One of these last patients was a 31 year old Negress who was found to have early latent syphilis in 1937 She received 5 doses

of mapharsen and 3 of arsphenamine, followed by a blood d crasia attributed to arsenic. During her seventh pregnancy 1938 she received 33 doses of bismuth before the pregnai terminated successfully in the delivery of non-syphilitic tripl who have remained well for 8 years. The mother's serolo test and cerebrospinal fluid were both negative at the end of t pregnancy. Subsequently she has been observed through th

TABLE I

The Stage and Duration of Syphilitic Infection in Mothers Previously Treated but Untreated During Subsequent Pregnancies

Diagnosis at Onset of Treatment For Syphilis	Number of Patients	Duration of Infection at the Time of Observation for Untreated Pregnancy		
		Less than 2 years	2 5 years	More than 5 years
Primary syphilis	94	24	43	48
Secondary syphilis	21			
Early latent	44	2	42	
Late latent	111		76	35
Asymptomatic CNS	9		9	
Late asymptomatic	8		8	
Congenital	48			48
Unclassified	28		28	

TABLE II

Maternal Treatment Prior to Pregnancy in Which no Treatment was Given and Maternal STS at Time of Untreated Pregnancy

Amount of maternal treatment prior to pregnancies during which no treatment was given	Number of Patients	Maternal blood serologic tests a onset of observed pregnancy		
		Negative	Doubtful	Pos tive
More than 4 gms	332			
2-4 gms	18	267	35	61
Uncertain but considered "adequate"	10			
Less than 2 gms	3			

ional pregnancies during which antisyphilitic treatment withheld The offspring of these three pregnancies have ed free of evidences of syphilis

e second woman who received "inadequate" treatment for ginal infection was a 21 year old Negress found to have dary syphilis in 1930 After 7 doses of arsphenamine and bismuth, she refused further treatment but became sero- ally negative Both blood and cerebrospinal fluid serologic remained negative during the successful pregnancy ob- d 15 years later

he third of the "inadequately" treated patients was 21 years when the diagnosis of late latent syphilis was made, and ved only 6 doses of arsenic before discontinuing treatment remained serologically negative throughout two observed nancies 6 and 11 years later, and her children were both nal

hese 363 previously treated women have been observed ugh 570 pregnancies during which maternal treatment was tted The results of these 570 pregnancies are outlined in e III There were 523 live births (91 6 per cent), 22 still- hs and 26 abortions (a fetal mortality of 8 4 per cent) This d birth rate is less than the normally expected figure for syphilitic pregnant women Pathologic examinations of the ducts of conception were made on 8 of the abortions and 12

TABLE III

Outcome of Pregnancies of Mothers Untreated During Observed Pregnancy

			Period of observation					Serial x rays of long bones* All negative
			Not seen	2 mos	2–4 mos	4–12 mos	1 yr*	
… pregnancies	570							
…ng children	523 (1 twin)		62	3	74	127	257	45
…hs	22	12						
…rtions	26	8						

* Only 10 per cent of the infants had serial x ray examinations because of the war time film shortage

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RESULTS OF TREATMENT OF OVER ONE HUNDRED CONTACTS OF PATIENTS WITH EARLY SYPHILIS WITH A 'ONE DAY ABORTIVE CURE'

By

Lee J Alexander, and *Arthur G Schoch*,
(Dallas, Texas)

For several years since the advent of the intensive therapy of syphilis particularly penicillin therapy, we have practiced and have advocated and urged the treating of the close repeater contact (marital and/or otherwise) who is found to be uninfected simultaneously with the infected partner in order (a) to prevent the contact from developing clinical syphilis, (b) to prevent reinfection in the original case—putting an end, to some degree to ping pong syphilis

In order for the treatment to be practical and usable, we realized that it must be simple and at the same time effective therefore we felt a one visit treatment, if one could be found would be ideal We knew that in order to prevent the development of clinical syphilis in the 'uninfected contact we had to cure subclinical syphilis of variable duration We felt that a one-day method of treatment might be devisable since in these cases we would be dealing with syphilis in its earliest form namely the incubation stage We knew one-day penicillin treatment of syphilis gave poor results, and that one-day arsenoxide therapy was not good Consequently, we decided to make use of a combination of the known good antispirochetal agents namely penicillin arsenoxide and oil soluble bismuth

TABLE I THE FORMULA

Penicillin in oil and beeswax, 900 000 units in one buttock (Abbott s Calcium Penicillin)
Bismuth Ethylcamphorate 3 cc (120 mg elemental bismuth) in the other buttock
Arsenoxide 0 05 to 0 06 gm intravenously

The time required for this treatment is approximately five minutes

Only persons who had recent exposures were used in th study Contact and source patients were cross-examined to mak certain of the exposure And if the exposure to primary ar secondary syphilis had occurred 2 or 3 months respectively pric to the interview with the source patient, the contact was no treated

One hundred and fifteen individuals who were negative, clin cally and serologically, but who had been exposed to patien who had darkfield positive lesions, were given the abortive trea ment Using the life-table method of illustration one sees th number of patients per each observation period and the occu rence of "so-called failures' During the entire observatio period 6 patients developed early syphilis—one, 14 months afte one, 11 months after, three, 4 months after, and one, 2 month after abortive treatment

TABLE II

Abort ve Treatment of Un nfected Contacts of Pat ents W th Infect ous Syph l s

TREATED GROUP RESULTS OF FOLLOW UP

Per ods of Observat on	Rx ed	1 Mo	2 Mo	3 Mo	4 Mo	5 Mo	6 Mo	7 Mo	8 Mo	9 Mo	10 Mo	11 Mo	12 15 Mo
Pat ents Per Per od	115	108	106	105	99	93	85	72	60	48	38	29	22
Re nfect on			1		3							1	1

The six patients who developed infectious syphilis are show in Table III Column 1 identifies the cases Column 2 shows th dates on which abortive treatment was given Column 3 show the dates at which infectious lesions were found and the typ of syphilis found Columns 4 and 5 show the results of the ep demiological investigations conducted with reference to eac incidence of clinical syphilis (column 3) Column 6 shows th results following treatment for clinical syphilis (column 3)

One hundred and thirty uninfected contacts were not treated

TABLE III

Patients Who Developed Infectious Syphilis Following Abortive Treatment

No	Abort. Rx	Reinf	Epidemiology		Observation Following Reinfection
46-2006 ELD	12-16-46	11-20-47 Neg L1	46-733 EMD	11-26-47 L11 ???	Neg @ 1 mo
47 413 De H.	3-4-47	5-27-47 Pos L1	Pickup 47-1091 42-2472	??? Neg Neg	Neg @ 10 mos
47 744 FJ M	4-11-47	8-14-47 Pos L1	47-1671	8 14-47 L11	Neg @ 5 mos
47-663 SF	4-8-47	8-26-47 Pos L1	Pickup	???	Neg @ 2 mos
46-2752 LB	11-20-46	1-20-48 L11	???	??? G C Oct 1947 Seroneg	??? Cooperation poor
47 530 EA.	3-17-47	7-22-47 Pos L1	47 1702 47-1704 47-1739	8-20-47 Pos L1 Neg 8-25-47 Pos L11 47 1146 6-11-47 Pos L1	Lost

Fifty four (41 5%) remained negative, and 75 (58 5%) developed clinical syphilis

Discussion

The immense value and necessity of treating uninfected contacts is obvious Actual disease (so far as we are able to detect it) is prevented This in turn eliminates spread to others

The question is · How effective is the abortive treatment? It seems to be, at the worse, just under 100% Some might argue that the 6 patients who showed clinical syphilis after abortive treatment are actual failures We like to think none of them

TABLE IV CONTROL GROUP

Negative and Remained Negative	*Negative and Became Positive*	TOTAL
54 (41 5%)	76 (58.5%)	130

is a failure Three certainly had been exposed to infectio syphilis after they had been given abortive treatment; a four patient acquired gonorrhea, received treatment for it, and thr months later had secondary syphilis (this came 11 months aft abortive treatment) Two patients named pickups We all kn the pickup is a mighty fertile source of infection

One certainly cannot overlook these figures—In the untreat group of 130 patients, 76 developed clinical syphilis: In t treated group of 115 patients, 6 developed syphilis

The abortive treatment is easy to administer and is safe

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LABORATORY DIAGNOSIS OF GRANULOMA INGUINAI AND STUDIES ON THE CULTIVATION OF THE DONOVAN BODY*

By

*Robert B Dienst***

At the present time there are three procedures available substantiate the clinical diagnosis of granuloma inguinale O procedure is to determine the presence of characteristic Donov bodies in the diseased tissue either by stained tissue smears by biopsy [1,2], another, to determine the presence of compleme fixing antibodies in the serum of the patient[1,4,5], and third, determine skin reaction of the patient to an intradermal inoc lation of Donovan body antigen[3,6] Of these tests, the exami tion of stained smears is the most accurate and certainly t simplest Exudate from the infected tissue when properly tak and properly stained demonstrates to an experienced exami characteristic intracellular micro-organisms which are speci for laboratory diagnosis of granuloma inguinale To our kno edge, there is no other micro organism with morphologic a staining characteristics to confuse with Donovan bodies as se in the cytoplasm of the large mononuclear cells always associat with exudate from lesions of granuloma inguinale Slide No

* Aided by a research grant from the Division of Research Grants a Fellowships National Institute of Health, United States Public Hea Service

** In collaboration with Robert B Greenblatt and Calvin H Chen at t University of Georgia School of Medicine Augusta, Georgia

... as the morphologic and intracytoplasmic characteristics of Donovan bodies When the organisms are not numerous in the infected cell, large and well defined capsular material is demonstrated, when however, the organisms are overcrowded in the cytoplasm, the capsules, though present, may not always be stained. Tissue smears stained with Wright's stain* are very satisfactory If the dye is allowed to remain on the smear for two and one half minutes before being diluted with distilled water, the capsular substance of Donovan bodies is deeply stained as demonstrated in Slide No 2 The micro-organisms with deeply stained capsules are more readily seen by the examiner The presence of extracellular organisms resembling Donovan bodies should always be regarded as suspicious in examining a specimen but final diagnosis should be made only when characteristic intracellular organisms are seen

In making a laboratory diagnosis of granuloma inguinale, one is impressed with the importance of properly made tissue smears The lesion should be thoroughly cleaned before the specimen is obtained Tissue smears taken from poorly selected areas may give a false negative result In typical clinical cases of granuloma inguinale, one tissue smear is often sufficient to demonstrate the numerous and characteristic intracellular Donovan bodies but in atypical, complicated and early lesions several attempts may be necessary before a positive specimen is obtained

Anderson in 1943 reported the cultivation of Donovan bodies in the yolk sac of chick embryo[8,7] The organism was shown by her to be antigenically related to the disease[3] Several investigators have since confirmed her reports on cultivation of Donovan bodies in yolk of developing chick embryos[2,9,10,11] We have been successful in isolating and cultivating Donovan bodies from three patients Our first strain was obtained from a typical ulcerating inguinal lesion of granuloma inguinale by a technique similar to that reported by Anderson and later by Beveridge[10] The procedure was laborious and successful isolation was more or less by chance[11] Our second isolation was obtained by recovering the Donovan bodies in pure culture from a Negro volunteer who submitted to subcutaneous transplantation of diseased tis

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* Wright s stain (National Aniline & Chemical Co)

Methyl alcohol (acetone free)	485 cc
Glycerine	15 cc
Wright s powdered dye	1 gram

sue The experimentally produced lesion was aspirated befor rupture and the organisms were grown in the yolk sacs of 5 day old chick embryos This is an interesting strain in that th inoculum of diseased tissue transplanted to the Negro voluntee was obtained by biopsy from a streptomycin resistant case o granuloma inguinale Our third strain was isolated from pu aspirated aseptically from an inguinal swelling on a patien with advanced genital lesions of granuloma inguinale Slid No 3 demonstrates the presence of encapsulated forms of Dono van bodies can be seen in a stained smear of infected yolk sack o chick embryo

The cultivation of recently isolated strains of Donovan bodie has been reported only in yolk sac or embryonic chick yolk In her studies Anderson concluded that some growth factor for Donovan bodies was produced in the yolk of developing chick embryos following the first 4 or 5 days of incubation[8] She failed to get growth in the yolk of unfertilized eggs and Donovan bodies reproduced in the yolk of inoculated fertile eggs only as the embryo developed on incubation

We have been successful in growing the organisms in fresh unincubated yolk medium The Donovan bodies multiplied in yolk medium prepared from unfertile eggs as well as from fertile eggs The ability of the plain yolk medium to support the growth of Donovan bodies of a freshly isolated strain depended on a physical property of the medium The addition of a very small amount of agar to the yolk medium apparently satisfied the growth requirement This observation of a growth factor for Donovan bodies is similar to that reported by Salvin[12] on the cultivation of *Histoplasma capsulatum* In his experiments Salvin concluded that a yeast phase of *Histoplasma capsulatum* could be obtained in artificial cultivation only in media contain ing a small percentage of agar silica gel oil or some similarly functioning substance

The media used to cultivate the Donovan bodies are prepared in the following manner Yolk is removed aseptically from fresh eggs and diluted 1 1 with sterile saline A base medium con taining 1% Bacto peptone 0 3 Bacto tryptone 0 3% dextrose and 0 2% sea salt is prepared and adjusted to pH 7 3 The base medium containing the desired percentage of agar is then steri lized and allowed to cool to 45 C before adding an equal portion of the diluted yolk In our preliminary experiments agar was added to the base medium so that with the addition of the sterile

diluted yolk in several experiments, no growth of Donovan boc occurred The Negro was susceptible to infection as an exp mental lesion was later produced on him by transplanted disea tissue

The ability to grow Donovan bodies in cell free medi greatly facilitates laboratory studies To be able to cultiv the organisms in fresh rather than embryonic yolk offers added advantage We have not had an opportunity at pres to attempt primary isolation of Donovan bodies in the y medium

CONCLUSIONS

The simplest and most reliable laboratory procedure for di nosis of granuloma inguinale is to determine the presence intramonocytic Donovan bodies in the tissue smear stained Wright's stain

Recently isolated strains of Donovan bodies can be cul vated in fresh yolk medium free from any growth factor pr duced by developing embryonic tissues

The addition of a small percentage of agar to fresh yo medium furnishes a factor essential for cultivation in vitro recently isolated strains of Donovan bodies

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THE EPIDEMIOLOGY OF GRANULOMA INGUINALE

By

*Geoffrey Rake**

I wish to discuss today certain developments in my laboratory which may throw light on the vexed question of the epidemiology of granuloma inguinale Let me begin by refreshing your memory as to the current state of our knowledge The disease although not confined to the Negro occurs predominantly in that race, the ratio to other races being 9 1 in number of cases When it occurs in members of races other than Negroes there is usually associated poor personal hygiene Although the disease is regarded as venereal, history of infection of the sexual partner, even in the face of frequent and repeated exposure, is very rare, moreover the lesion tends to be rather in the anogenital region than on the genitalia There are papers in the literature particularly from the South Pacific suggesting that under rare conditions the disease may become almost epidemic with the most disastrous results, but such events scarcely concern our discussion of the disease as it occurs in the United States

A great advance in our knowledge of granuloma inguinale came with the isolation of the etiological agent in the embryonated chicken egg by Katherine Anderson She was able to carry out both complement fixation tests and skin sensitivity tests with her material Subsequent work in my laboratory has resulted in the adaptation of Anderson s agent *Donovania granulomatis*, to artificial media and antigens have been obtained which are free from any egg material With such antigens 197 sera have now been tested Table I contains a summary of the

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TABLE I

Complement Fixation Tests Using Antigens Prepared from *Donovania Granulomatis*

Diagnosis	Number of Cases Positive	Number of Cases	Per Cent of Cases Positive
Granuloma Inguinal	55/63		87
Venereal Exposure	8/71		11
Chronic Ulceration	4/29		14
Tuberculosis	0/10		0
Normal (Disease-free)	0/24		0

results obtained with these sera according to their sourc and it will be noted that 87% of sera from cases diagnosed a granuloma inguinale gave specific fixation with the antige prepared from the strain grown on artificial media Thi figure compares with positive fixation obtained in 92% of ser from such cases when the chick egg antigen was used It wi also be noted from the slide that no fixation occurred in 24 ser from normal disease-free individuals or in 10 sera from individ uals with tuberculosis On the other hand positive results wer obtained in 11% of individuals suffering from venereal disease other than granuloma inguinale, and 14% in individuals witl extensive chronic superficial ulcers—either decubitus or varicose At the time when these figures first became available to us i was felt that the 11% of positive reactions in individuals suffer ing from other venereal diseases might be explained in part o mistaken diagnosis and in part on the possible existence of laten infections This latter explanation has been advanced elsewher for the more numerous cross fixations seen in the sera from venereally exposed individuals when the antigen of lympho granuloma venereum is used The 14% positive sera seen in individuals with chronic ulcerations was however more puzzling After considering the matter carefully, it was decided that it might be due to some antigen held in common both by a secon dary bacterial invader of such chronic ulcers and by *D granu lomatis* In view of certain morphological appearances, which will be discussed more fully below, it was felt that *D granulo matis* might prove to have closer relationships within the tribe *Eschericheae* than amongst other bacteria As a result antigens were prepared from several members of this tribe in a manner similar to that used for preparing the antigen of *D granulo matis* from culture on artificial media The species so selected






TABLE II

Complement Fixation Tests Using Antigens Prepared from *K Rhinoscleromatis*, *K Pneumoniae* *E Coli* and *A Aerogenes*

	K Rhinoscleromatis	*K Pneumoniae*	*E Coli*	*A Aerogenes*
No of sera tested	47	68	55	55
% of overall correlation with *D Granulomatis* antigen	94%	78%	69%	64%
% of correlation with sera positive with *D Granulomatis* antigen	89%	63%	45%	36%
% of correlation with sera negative with *D Granulomatis* antigen	100%	100%	100%	100%

were *K. rhinoscleromatis*, *K pneumoniae*, *E coli* and *A aerogenes* These antigens were then tested against as many of the sera used for the test with the antigen of *D granulomatis* as were still available The results of these tests are seen in Table II It will be noted from this table that the correlation between the results obtained with the antigen of *K rhinoscleromatis* and *D granulomatis* were reasonably close The antigen of *K pneumoniae* gave positive results in 63% of sera positive with the antigen of *D granulomatis* while the antigen of *E coli* gave fixation with approximately one half of the sera and that from *A aerogenes* with approximately one third of the sera These results seem to us to indicate that *D granulomatis* probably is a member of the tribe *Eschericheae* and is most closely related to the genus *Klebsiella* in this tribe That it is the causative agent of at least the majority of cases of granuloma inguinale is shown by the high proportion of sera from such cases which give positive fixation against it whether cultured in the yolk sac or after adaptation on artificial medium

When one turns to the electron microscope studies of this organism, whether growing in the yolk sac of the chicken egg or on artificial medium, one is struck by two features The first is the high degree of pleomorphism to be seen in these organisms and that is brought out in the next lantern slide The second point is that the typical forms of *D granulomatis* resemble very closely those of *K pneumoniae* which we have examined This is shown in the next lantern slide

The results of these studies leave one therefore with the following facts *D granulomatis* seems to be related to members of the tribe *Escherichеae* This relationship can be demonstrated not only by an investigation of antigenic pattern but also by studies on morphology As you know, many members of the *Escherichеae* are common fecal organisms Furthermore, there is evidence from our own studies that *D granulomatis* represents an organism which has become highly adapted to animal tissues, not as much, it is true, as has the bacillus of leprosy, but to a degree approaching that Even after growth has been obtained in the fertile chick's egg it is with certain amount of difficulty that adaptation to artificial media has been carried out, and as we have pointed out elsewhere, even after many passages on artificial medium the growth is delayed and scanty, although such strains immediately grow out flourishingly when reintroduced into the chick egg

That the causative agent, *D granulomatis*, might be an organism derived from the feces is no new idea since it was suggested by DeMonbreun and Goodpasture fifteen years ago It seems perhaps probable that we are dealing with an organism with a high degree of mutability, some strains of which once established in the human tissues are not cultivatable except by some such method as that of Anderson's using the chicken embryo Other strains, perhaps less highly adapted, might account for the infrequent descriptions of cultivation of organisms which grow poorly and do not survive many transfers such as those described by DeMonbreun and Goodpasture, while still less highly adapted strains might account for the organisms of Souza Araujo and others who have repeatedly claimed the isolation from cases of granuloma inguinale of strains related to the genus of *Klebsiella* which grow reasonably well on artificial media If one accepts the evidence that *D granulomatis* is the cause of *granuloma inguinale* and further accepts the hypothesis that this organism is related to the genus *Klebsiella* and is a fecal organism of high mutability some of which mutants have strong intracellular tropism some of the unexplained facts concerning the epidemiology of this disease become clear The disease occurs in individuals whose personal hygiene is low and in regions of the body likely to be constantly contaminated with fecal material The origin could then be explained on the infection of small traumatic lesions produced on and around the genitalia during sexual intercourse or even in lesions due pri-

mainly to some other specific venereal disease agent which becomes overgrown The usual absence of infection in the sexual partner would not be unexpected under such circumstances On the other hand, the hypothesis of high mutability could also be extended, without distorting it unduly, to include those described *epidemics* of the disease which apparently occur from time to time

I trust that you will accept this summary of the work done in my laboratory, together with certain hypotheses built upon it, with forbearance I realize that it is unwise for an individual whose work is largely within the four walls of a laboratory to embark upon hypotheses such as those I have outlined to you today However, if in so doing I have stimulated your interest in the results we have obtained and directed your thoughts along certain channels which those results seem to me to open up, whether my general hypothesis proves to be correct or not, I shall be very content with what I have achieved

EXPERIMENTAL PINTA

By

Francisco Leon Blanco
(Habana, Cuba)

The finding of treponemes in the skin and lymph nodes of a Cuban case of pinta by Alfonso, Grau Triana and Leon Blanco[1] prompted experimental study to determine if this treponeme was a new species, or *T pallidum* or *pertenue* This work was carried out by the present author in Mexico where more than 300,000 pinta patients were available

Between October 1938 and May 1942, I demonstrated the presence of treponemes in the skin lesions of *2 000 cases* of Mexican pinta, as well as the absence of *such microorganisms* in the healthy contacts living in the same region[2] Between October 1938 and December 1939 a series of experimental inoculations were carried
discovered by the
previously known
or primary lesion of pinta and the lesions of
(pintids) until then unknown The clinical evolution of pinta was thus described[3]

My experimental work was later confirmed by Gomez Farias[4] in Mexico, Oteiza[5] in Cuba and Padilha Goncalves[6] in Brazil

HUMAN EXPERIMENTAL PINTA

The first experimental transmission of pinta from man to man was carried out on myself[4] and later in 27 volunteers To these 28 cases must be added 2 of Gomez Farias[4], 12 of Oteiza[5] and 9 of Padilha Goncalvez[6], making a total of 51 known experimental inoculations of pinta in human beings

These 51 cases may be divided in five groups:

(1) 26 cases had not suffered from pinta or syphilis before
(2) 4 cases had suffered from pinta and had been cured for some time before reinoculation was attempted
(3) 5 cases were suffering from late pinta at the time of reinoculation, their serologic tests were strongly positive and abundant treponemes were demonstrated in their skin lesions before reinoculation
(4) 9 had suffered from syphilis, insufficiently treated and their serologic tests were strongly positive at the time of inoculation
(5) 9 were afflicted with yaws, and their serologic tests were strongly positive at the moment of inoculation The duration of the yaws infection was between 4 and 11 months None had received treatment The results obtained in these groups varied and must be stated separately

GROUP I —*The Primary Stage* —Of the 26 cases of this group 14 were inoculated with serous exudate rich in *Treponema carateum* from the late cutaneous lesions of pinta patients, 8 were inoculated with material from early pintids and 4 were inoculated with exudate from experimental primary lesions

Inoculations were carried out intracutaneously with the serous exudate diluted in saline solution in 17 cases, percutaneously depositing the exudate on the abraded skin, in 9 cases In 3 of the latter, inoculation had been previously tried unsuccessfully before by depositing the infective exudate on the healthy non abraded skin In 17 patients only one inoculation was performed, in 3 two inoculations and in 6 three simultaneous inoculations were performed

The results were positive in all 26 volunteers Each inoculation site developed an initial lesion, so that 17 had only one lesion, 3 had two lesions and 6 had three initial or primary lesions, all of which followed parallel development and course

The period of incubation varied between 3 and 61 days, usually between 6 and 14 days.

The first manifestation after inoculation appears as a lenticular erythematous spot on the skin, but this is a fleeting symptom, observed usually at 24 hours, and disappearing after 72 hours This may be a traumatic or transitory response to the injected fluid Later appears a papule the size of a pinhead, or a lenticular maculo-papular erythema. There may be 2 or more papules close to each other. These lesions grow slowly but steadily in size, reaching maximum size and elevation about the 15th to the 20th day Generally they are oval or semispheric in shape, infiltrating the dermal tissues, slightly squamous or lichenoid, pink in white skins and violaceous in dark skins, and have a *corona* of scales in their periphery These scales may accumulate on the surface in neglected cases, become stratified and resemble the clinical aspect of late nodulo-squamous syphilid On removal, these scales leave a glistening red surface with ruby-red puncta, corresponding to the dilated capillaries of the papillae Around the papula and separating it from the healthy skin there may be an erythematous halo from 2 to 3 mm in width and sometimes very fine radiating creases

The initial lesions may continue increasing in size by peripheral extension and by the coalescence and fusion of adjoining lesions, and about the 60th day may reach about 2 cm in diameter

The initial lesion may remain as the sole manifestation of pinta for 3 months to a year The clinical aspect may be that of a psoriasiform erythematous patch which may resemble ringworm of the skin, lichenoid eczema or parakeratotic chronic dermatitis Often the main lesion is surrounded by smaller satellites which may finally coalesce and become incorporated into the larger lesion Pruritus is a fairly constant symptom and as the result of scratching, secondary infected ulcerations may occur The initial lesion of pinta never undergoes spontaneous ulceration

The neighboring lymph nodes are *slightly* enlarged, hard, movable, and [illegible] by puncturing these glands shows [illegible] ntal proof that in

The seconda [illegible] od before the ap-

pinta, treponemes occur in the circula[illegible] lesions, but their pres-

pearance of the disseminated cutaneous lesions, but their pres-

ence in the lymph nodes points *to their* dissemination through

the lymphatic system It is logical to believe that they may be carried by the lymphatic and the venous circulation to the peripheral cutaneous vessels where they become localized, multiply and cause the lesions which I described under the name of "pintids"

Experimental infections in volunteers have allowed us to ascertain how long after inoculation these pintids make their appearance In my study four cases were followed until pintids appeared on the skin, which were respectively 97, 112, 152 and 142 days In the case reported by Gomez Farias[4] the disseminated lesions showed at the end of 85 days In Oteiza's cases[5] the outbreak of pintids appeared after 88, 133, 139 and 142 days The average interval is about 126 days, with a minimum of 85 and a maximum of 193 days

Pintids appear first as a discrete roseola, the lesions being small, lenticular, pinkish in color, non elevated, covered with apparently normal epidermis There may occur papular lesions of military size, infiltrating the corium, pink or violaceous and slightly scaly The number of lesions vary from a dozen to about one hundred, there may be successive outbreaks These lesions do not fade and disappear, as in syphilis, but become permanent and enlarge gradually, spreading and coalescing to form larger patches with an erythemato squamous surface, or one which may be psoriasiform, annular, erythemato-pigmented or in parts achromic Some pintids resemble the circinate, annular or psoriasiform syphilids They may last for months and years covering by gradual extension a large part or most of the surface of the skin I could follow these pintids on myself for the best part of three years, during which the lesions changed color and clinical aspect several times None of my own pintids disappeared spontaneously However, in two volunteers inoculated by Leon Blanco and Oteiza in 1944, the initial lesion as well as the secondary pintids have disappeared without treatment, although the serologic tests remain strongly positive

In all cases the serologic tests for syphilis are negative during the long period of solitary initial lesion, but as soon as the first outbreak of pintids occur, these tests become positive in a few days Specific treatment similar to that of syphilis may change them to negative

GROUP 2 This group was composed of 4 volunteers who had had pinta and had been successfully treated with specific treat-

ment, three of these patients were inoculated by myself[3] and one by Oteiza[5]

Of my cases, one had been previously experimentally infected, treated and apparently cured within 60 days after the inoculation when the serologic tests were still negative and no pintids were yet visible. Another patient had had pinta for 10 years, and had been treated and apparently cured 4 years before this experiment. The third patient had acquired pinta one year before At the time of this experiment he had 14 pintids, and positive serologic tests, but was treated and apparently cured with neo arsphenamine Oteiza s case had been inoculated in 1940, and treated two years later during the secondary pintid period, with reversal of the serologic tests

Fresh inoculations in these 4 cases resulted in the appearance of typical initial lesions at the sites of inoculations after 7, 20, 22 and 31 days respectively, with the same characters as observed in Group 1 Oteiza s case was observed during 6 months showing a shower of pintids on the skin after 142 days Serologic tests again turned positive in this case

GROUP 3 This group was composed of 5 volunteers suffering from pinta at the moment of the experimental inoculation All had positive serologic tests and all harbored *Treponema carateum* in the existing cutaneous lesions All these patients were inoculated intracutaneously with infective serum containing abundant *Treponema carateum* the inoculations being made on apparently normal skin After 49 days no lesions appeared at the sites of inoculations

GROUP 4 This group included 10 patients with syphilis who had been insufficiently treated had strongly positive serologic tests but no cutaneous lesions at the time of inoculation with pinta material

Three of these patients were inoculated by myself 6 by Oteiza and one by Gomez Farias In cases the patients had acquired syphilis 3 years 14 months and 4 months respectively before the moment of this experiment Oteiza's cases had a history of syphilis and strongly positive blood tests Gomez Farias' case had been treated 4 years before and his serologic tests were negative My three cases inoculated with pinta material developed typical initial pinta lesions at the site of inoculation 12 17 and 20 days respectively after the inoculation These initial lesions although smaller and slower in evolution were identical with those observed in non syphilitic patients One case was followed for 5 months and presented an outbreak of pintids 127

days after inoculation Another volunteer inoculated with exudate taken from these pintids acquired an initial pinta lesion, not a syphilitic chancre Of the 6 patients inoculated by Oteiza the experiment failed in 5 after 9 months of observation, one developed an initial pinta lesion 12 days after inoculation In Gomez Farias' case an initial pinta lesion appeared 7 days after inoculation

GROUP 5 This group consisted of 9 untreated patients affected with yaws, who had contracted the disease from 3 months to 4 years before the experimental pinta inoculation Two had latent yaws, 7 had active lesions All were inoculated intracutaneously Of these 9 inoculations, two were positive One with primary and one with latent yaws developed initial lesions of pinta after incubation periods of 32 and 35 days respectively

SUMMARY

This article reviews the experimental inoculations performed with pinta material in 26 cases published up to the present

The initial lesion of pinta is a papule which appears from 3 to 61 days (average 14-20 days) after intracutaneous inoculation

Reinfection on treated pinta was successful in 4 cases The initial lesion was identical to that seen in individuals not previously infected The incubation period in these cases was 7 20, 22 and 31 days respectively One of the patients followed during 6 months had an outbreak of pintids on the 142nd day

Superinfection could not be obtained experimentally in cases with late manifestations of pinta In five cases attempted all gave negative results

Of 9 inoculations with pinta material in patients suffering with latent seropositive syphilis 4 were successful In an early case of syphilis intensively treated and with negative serologic tests an initial lesion of pinta was obtained on the 7th day after inoculation with pinta material Only 4 of 9 inoculations were positive in patients with latent seropositive syphilis, seems to point to partial cross immunity

Pinta patients do not become immune As soon as the infection is cured by specific treatment, reinoculation is possible experimentally, whether the disease remained in the initial stage or advanced to the secondary period of disseminated skin manifestations

There is no difference in experimental initial lesions, whether the subjects have previously suffered from pinta, syphilis, or yaws, or have had no earlier spirochete infection

The initial lesion of pinta is different from those of syphilis and yaws

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NON-GONOCOCCAL URETHRITIS IN THE MALE

By

Charles M Carpenter[1]

and

Lt Col Raymond P Hughes[2]

Non gonococcal urethritis became a significant entity during the war years Not infrequently, a stigma was associated with the record of a man in the Armed Forces who had gonococcal urethritis On the other hand, if the urethritis was of non-gonococcal origin the individual's sexual conduct was seldom questioned The development of the cultural method for the diagnosis of gonorrhea focused attention on non gonococcal urethritis and aided in the differentiation of the "specific" from the "non specific" infection

Until comparatively recently the majority of physicians treat-

[1] C[illegible] Ba[illegible]ology to the Office of the Surgeon General,

[2] C[illegible]nch *Medical Consultant's* Division,

ing venereal disease believed that practically every case of urethritis in the male was a *Neisserian* infection Prior to the use of cultures for detecting the gonococcus the failure to find the organism in films from urethral exudates was considered to be inconclusive evidence that the gonococcus was absent Al though the present cultural method is not infallible confidence in its use has increased because it has been demonstrated repeat edly that the cultural method is superior to the microscopic examination of Gram stained films

The therapeutic efficacy of penicillin in gonorrhea has aided the characterization of non gonococcal urethritis From 90 to 95 per cent of patients are cured with a single course of therapy Thus when a patient with urethritis fails to respond to peni cillin it is most unusual Reports of penicillin resistant gonor rhea have been published[1 3] but in our experience with several hundred alleged cases of this type no such evidence was ad duced[4] To date no strain of the gonococcus has been observed *in vitro* to grow in a concentration of penicillin greater than 0 08 unit per ml of medium Reinfection was responsible for many of the so called failures In other instances of alleged gonococcal urethritis which did not respond to therapy with penicillin either a species of *Neisseria* other than the gonococcus or bac teria belonging to entirely different genera were isolated These observations confirm the prevalence of a urethritis of non gono coccal origin

The syndrome presents for study its etiology, epidemiology prevention and treatment Of special interest is determination whether the disease is of venereal origin Most of the observa tions to date have been made on Army personnel in the Pacific Theatre and in the First and Third Army Areas among whom was a small group of debarkees from the Mediterranean Theatre Limited observations have been made among civilians

Procedure

From 100 to 200 men were awakened each morning well be fore reveille and marched to the dispensary Strict precautions were taken to prevent them from urinating prior to examination The external genitalia were examined for urethral discharge penile ulcers and epididymitis The urethra was carefully stripped by the examiner and the lateral margins of the meatus were everted in order to examine the mucous membrane for inflammatory changes The inguinal nodes were palpated for evidence of lymphadenopathy Men with urethritis were with

drawn from the line and retained for bacteriologic examination Care was taken to exclude men with a mucoid urethral exudate resulting from alleged sexual exposure the previous night or men who had seminal fluid in the urethra following a nocturnal emission Only those cases who had a definite mucopurulent or purulent exudate were classified as urethritis In some cases the exudate was scanty and would have been readily overlooked had they not been observed prior to urination

The following technique for making cultures was carried out The meatus was cleansed with a sterile gauze sponge moistened with 95 per cent alcohol A drop of the urethral exudate was collected on a sterile cotton tipped applicator and streaked directly onto the surface of a chocolate agar plate After a little experience it was possible to distribute the inoculum on the medium so that individual colonies could be studied Bacteriologic examinations were also made of specimens from the urethra of 10 apparently normal men in each group of 100 to compare their bacterial flora with that of men presenting symptoms of urethritis Cultures were likewise made from the sediment of first-voided urine collected from 50 medical students who clinically showed no evidence of urethritis

All cultures were incubated for 48 hours at 36° C in an atmosphere with an increased carbon dioxide tension The examination of the cultures for the gonococcus and their final identification were made according to standard procedures[5] In the majority of cases only a single film and culture were made but in several groups cultures were made on from two to four different media suitable for the isolation of the gonococcus Furthermore repeated cultures were often taken to carefully check the procedure Chocolate agar with either Supplement A or B Difco was employed as the basic medium Films were stained by Hucker s modification of Gram s stain The bacteriologic examination was not limited to the detection of the gonococcus Special attention was given to the predominating species recovered in an attempt to determine their relationship to the urethritis

This report is concerned primarily with the prevalence of non gonococcal urethritis Considerable bacteriologic work has been carried out but as yet the agent or agents responsible for the syndrome have not been established

RESULTS

A survey of 4 190 men examined for evidence of urethritis revealed that 60 (1 4 per cent) had gonococcal urethritis w

TABLE I

Urethritis Among 4 190 Men in U S Army

Source	TOTAL					WHITE					NEGRO				
	No Men Exam	Cases of				No Men Exam	Cases of				No Men Exam	Cases of			
		Gonococcal Urethritis		Non-Gonococcal Urethritis			Gonococcal Urethritis		Non-Gonococcal Urethritis			Gonococcal Urethritis		Non-Gonococcal Urethritis	
		No	Percent	No	Percent		No	Percent	No	Percent		No	Percent	No	Percent
Pacific Theatre	1800	22	1 2	223	12 4	646	2	0 3	43	6 7	1154	20	1 7	180	15 6
Zone of Interior	2390	38	1 6	180	7 5	944	2	0 2	34	3 6	1446	36	2 5	146	10 1
Grand Total	4190	60	1 4	403	9 6	1590	4	0 25	77	4 8	2600	56	2 2	326	12 5

403 (9 6 per cent) had non-gonococcal urethritis The number of cases of the two types of urethritis among 1,590 white men were gonococcal urethritis 4 (0 25 per cent), non gonococcal urethritis 77 (4 8) per cent) For 2,600 Negroes, the distribution was gonococcal urethritis 56 (2 2 per cent), non gonococcal urethritis 326 (12 5 per cent) A comparison of the two infections among 1,800 men overseas revealed that 22 (1 2 per cent) had gonococcal urethritis and 223 (12 4 per cent) had non-gonococcal urethritis In the Zone of Interior, 38 (1 6 per cent) had gonococcal urethritis and 180 (7 5 per cent) had non gonococcal urethritis In the case of racial distribution in the two areas the following data were obtained Pacific Theatre, among 646 whites 2 (0 3 per cent) had gonococcal urethritis, and 43 (6 7 per cent) showed evidence of non gonococcal urethritis, among 1,154 Negroes 20 (1 7 per cent) showed gonococcal urethritis and 180 (15 6 per cent) non gonococcal urethritis In the Zone of Interior the racial distribution was as follows Of a total of 944 white men, 2 (0.2 per cent) had gonococcal urethritis and 34 (3 6 per cent) non gonococcal urethritis Among 1,446 Negroes, the cases of gonococcal urethritis were 36 (2 5 per cent) and of non gonococcal urethritis 146 (10 1 per cent) (Table I)

Sixteen (8 0 per cent) cases of urethritis were detected among 200 debarkees from the Mediterranean Theatre, even though this group had been subjected to several recent inspections prior to our examination Of this number, 7 (3 5 per cent) were of gonococcal origin and 9 (4 5 per cent) were non specific in character Surprisingly, the examination of another group of 200 new enlistments, the youngest men in the study who were awaiting assignment for their basic training, half of whom were white and half colored, revealed 18 cases (9 0 per cent) urethritis Five (5 0 per cent) were among white men, and 13 (13 0 per cent) among Negroes No gonococcal infection was detected, however

Facilities and personnel were not available to carry out complete bacteriologic examination on each patient The following observations were made, however *Streptococci* were isolated from the urethral exudates of 153 (38 per cent) of 403 men with non gonococcal urethritis *In* 77 (19 per cent) the streptococcus was the *predominant organism* Two types were recovered (1) the Alpha type *forming* greenish zones about the colonies on blood agar and (2) the Gamma type which produced no *hemolysis* Several strains of the Alpha type were identified as

Streptococcus fecalis The frequency with which streptococci were detected varied considerably from group to group Diphtheroids and staphylococci were not infrequently recovered in culture as the predominant organism In some instances they grew concomitantly with streptococci In a few cultures, small Gram-negative bacilli appeared, but their final identification was not possible at the time the survey was in progress

The cultures from the urine sediment of 50 medical students and from 40 men recently returned from the Pacific Theatre to the Zone of Interior, as well as from 200 men in the Pacific Theatre, with no clinical evidence of urethritis revealed findings somewhat similar to those obtained on the men with non-gonococcal urethritis Streptococci were present in culture from 78 (27 per cent) of the men In numerous cases only a few colonies were present, but not infrequently a profuse growth of the streptococcus occurred

Discussion

The intensified interest in non-gonococcal urethritis in the male and its differentiation from alleged penicillin-resistant gonorrhea has emphasized the need for further study of the entity The prevalence of urethritis among sexually-active young men suggests that non-specific urethritis is acquired from sexual contact A few men with the disease maintained that they had never had sexual intercourse with women, however Although reports by several urologists have implicated a focal infection of either the prostate and/or the seminal vesicle as the cause of the urethritis, it was evident as a causative factor in only a few of the patients observed in this survey

The results of the bacteriologic examinations are not significant in the etiology of urethritis because of the similarity of the bacteria isolated from the urethral exudate of the men suffering from non gonococcal urethritis with the bacterial flora of the urethra of men considered to be normal Reports of a filterable virus[6] as a possible cause of non-gonococcal urethritis among Australian troops has stimulated further search for such an agent, but as yet it has not been observed to be the etiologic factor in this country Pleuropneumonia-like organisms have also been described as another causative factor[7,8] Such organisms have been isolated from the urethral discharge of men with urethritis and from the lower birth canal Whether they constitute a part of the normal bacterial flora of the genito urinary tract is as yet not known Further studies of the etiology, epi-

demiology, and treatment of non gonococcal urethritis are in progress by several investigators in this country which will no doubt aid materially in controlling the disease When the cause of the disease is well understood and the mode of infection is established the prevention and treatment can then be more effectively carried out

Summary

1 An examination of a total of 4 190 men in the Army, 1,800 from the Pacific Theatre and 2 390 from the Zone of Interior showed that 463 (11 0 per cent) had urethritis Sixty cases (1 4 per cent) had gonococcal urethritis and 403 (9 6 per cent) had non gonococcal urethritis

2 A comparison of the bacterial flora of the urethral exudate of 403 men with non gonococcal urethritis with that of the urethra of 290 normal men revealed, exclusive of the gonococcus, a similarity of organisms in both groups Streptococci were present in a greater number of cultures than any other organism and furthermore they were predominant in more instances On the other hand, streptococci were isolated from 38 per cent of the men with urethritis and from only 27 per cent of the normal men Special examinations were not made for pleuropneumonia like organisms nor for viruses

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HUMAN BLOOD LEVELS FOLLOWING VARIOUS DERIVATIVES OF PENICILLIN SUSPENDED IN VARIOUS ABSORPTION-DELAYING VEHICLES

By
D K Kitchen, E. W. Thomas, R. H Lyons, M. J Romansky, and C R Rein†

Buckwalter and Dickison[1] have recently reported a new repository type of penicillin product * They have introduced the principle of suspending penicillin salts in peanut oil gelled with aluminum stearates, water repellent substances Their study was an animal comparison of the absorption delaying properties of this vehicle with the conventional repository vehicles The study recorded here was designed to carry this comparison through the human subject

The facilities of four assay laboratories were used and assays were performed by two different methods, a modified Rammelkamp method and a cup plate procedure employing sarcina lutea Control blood samples were drawn before the injection, each patient then received 300,000 units of penicillin in repository form and consecutive blood samples were taken at the intervals indicated on Chart A The lines on the chart are the least squares fits to the logarithm of the average blood concentration with time in hours

Table I presents, in accordance with standard practice[2,3], the percentage of all patients whose blood concentrations were 03 units per cc or above at the indicated hours for the various products studied It also shows the total number of patients whose blood samples were assayed and the average concentration of penicillin per cc of serum at these various intervals

Table II shows the number of lots of each product tested and the percentage of particles of each product above or below a diameter of fifty microns on a relative weight basis

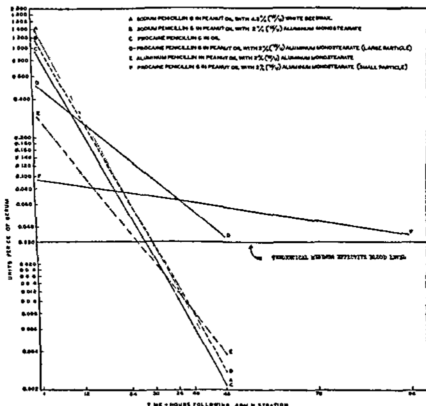
The results obtained by a single injection indicated that the

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* FLO CILLIN generously supplied by Bristol Laboratories Inc Syracuse N Y

† From Bristol Laboratories Syracuse N Y (Dr Kitchen) Bellevue Hospital and Ne[illegible] Y
(Dr [illegible] 19)
Antib[illegible] [illegible]tal
Wash[illegible] [illegible]ork
Unive[illegible]
W[illegible] [illegible]stol
Labor[illegible]

CHART A



gelled product warranted a series of observations following its prolonged use.

Table III exhibits blood concentrations following prolonged administration of procaine penicillin in oil gelled with 2% aluminum monostearate (large particle). Sixteen patients were injected once daily for 16 days and blood levels were determined at regular intervals for the first 24 hours and at 24 hour intervals thereafter until the concentration fell below .039 units per cc. following the last injection. The purpose of this study was twofold: 1) to observe any untoward effects of repeated injection; 2) to record maintenance of high blood concentrations.

DISCUSSION

The absorption of all salts of penicillin tested was delayed longer in the aluminum monostearate-peanut oil gel than when the same salts were suspended in peanut oil alone or in peanut

TABLE I

Number of Patients, Average Blood Levels and Per Cent of Patients with Blood Concentrations of 03 Units or Higher at Successive Time Intervals Following Single Injections (300 000 Units Each) of Six Repository Penicillin Preparations
(Sarcina lutea technic)

Time in Hours Following Administration	A Sodium Penicillin G in Peanut Oil With 4 8% (w/v) White Beeswax			B Sodium Penicillin G in Peanut Oil With 2% (w/v) Aluminum Monostearate			C Procaine Penicillin G in Oil		
	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher
1	17	1 032	100 0	18	2 583	100 0	81	1 120	100 0
12	20	1 310	100 0	16	140	87 5	80	190	78 8
24	22	077	77 3	16	038	62 5	78	050	42 3
30	17	035	58 8	17	022	23 5	75	019	18 7
36	17	021	41 1	18	013	27 8	75	010	12 0
40	13	004	0 0	18	010	16 7	75	006	12 0
48	19	002	5 3	18	004	11 1	75	003	4 0

TABLE I (continued)

Time in Hours Following Administration	D Procaine Penicillin G in Peanut Oil With 2% (w/v) Aluminum Monostearate (Large Particle)			L Aluminum Penicillin in Peanut Oil With 2% (w/v) Aluminum Monostearate			F Procaine Penicillin G in Peanut Oil With 2% (w/v) Aluminum Monostearate (Small Particle)		
	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher	Number of Patients	Average Blood Level	Per Cent of Patients With Blood Level 03 or Higher
1	160	593	98 7	39	251	100 0	59	117	98 3
12	162	244	98 1	45	083	88 9	60	080	76 7
24	162	120	90 1	47	067	55 3	60	075	93 3
30	161	092	82 6	39	035	33 3	60	068	90 0
36	159	069	75 5	43	013	16 3	59	057	84 7
40	160	050	63 8	39	002	2 6	59	051	84 7
48	161	038	52 2	46	006	8 7	59	056	88 1
72							39	048	79 5
96							28	039	75 0

TABLE II

Product	No of Lots	Particle Size
A— Sodium Penicillin G in Peanut Oil with 4 8% (w/v) White Beeswax	1	57% over 50 micron
B— Sodium Penicillin G in Peanut Oil with 2% (w/v) Aluminum Monostearate	1	50% over 50 micron
C— Procaine Penicillin G in Oil	3	78 8% over 50 micron
D— Procaine Penicillin G in Peanut Oil with 2% (w/v) Aluminum Monostearate (Large Particle)	3	93% over 50 microns
E— Aluminum Penicillin in Peanut Oil with 2% (w/v) Aluminum Monostearate	2	70% over 50 microns
F— Procaine Penicillin G in Peanut Oil with 2% (w/v) Aluminum Monostearate (Small Particle)	1	100% under 5 microns

TABLE III

89% OF PARTICLES OVER 50 MICRONS (Relative Weight Basis)

72 1035 (303 000 units of procaine penicillin per cc of peanut oil gelled with 2% aluminum monostearate)

Patient No.	24 hr	2nd d. 24 hr	3rd d. 24 hr	4th d. 24 hr	5th d. 24 hr	6th d. 24 hr	7th d. 24 hr	8th d. 24 hr	9th d. 24 hr	10th d. 24 hr	11th d. 24 hr	12th d. 24 hr	13th d. 24 hr	14th d. 24 hr	15th d. 24 hr	16th d. 24 hr	48 hr after last injection
1	<.039	.039	039	<.039	.078	.078	.078	078	078	039	078	078	078	039	078	039	.039
2	156	156	.078	078	078 156	312	156	312	312	156	078	156	312	.078	.078	.312	<.039
3	.078	078	.078	.078	156	078	078	156	078	.078	156	156	078	156	156	156	078
4	156	078		156	.312	078	156	156	078	312	078	156	078	078	156	.078	<.039
5	.078 156	156	078	078	156	312	.078	078	039	.078	039	156	.312	156	.078	.078	
6	078 156	.078	156	078	.078	156	078	078	156	078	156	156	.039	.156	.078	.078	.039
7	156	156	156	.078	156	312	312	312	078	.312	.312	.312	156	156	156	.312	trace
8	.039	.039	.078	.078	.078	.078	156	078	.078	078	.312	.078	.078	.078	.039	078	<.039

91% OF PARTICLES OVER 50 MICRONS (Relative Weight Basis)

72 1050 (303 000 units of procaine penicillin per cc of peanut oil gelled with 2% aluminum monostearate)

Patient No.	24 hr	2nd d. 24 hr	3rd d. 24 hr	4th d. 24 hr	5th d. 24 hr	6th d. 24 hr	7th d. 24 hr	8th d. 24 hr	9th d. 24 hr	10th d. 24 hr	11th d. 24 hr	12th d. 24 hr	13th d. 24 hr	14th d. 24 hr	15th d. 24 hr	16th d. 24 hr	48 hr after last injection
9	.039	156		.039	156	156	156	.312	312	.312	312	156	.312	156	312	.312	trace
10	.078	078	156	078	156	156	.039	.078	078	156	.078	078	.078	.078	078	.039	<.039
11	.078- 156	156	039	.039	078	.078	.078	.078	.078	156	039	078	156	.078	.078	.078	
12	.425	.312	.312	.312	.312	.312	.312	.312	.425	425	.312	156- .312	312	.312	.312	.312	.039
13	156	156	078	078	.078	156	.078	156	078	.078	078	156	156	156	.078	156	<.039
14	.078	.078	.312	156	.078		156	156	.425	.312	.312	156	156	156	156	.312	trace
15	trace	.078	.078	.312	156	.078	.078	.078	.078	.039	trace	039	156	.078	.078	.078	<.039
16	<.039	<.039	.078	.039	.039	.039	156	156	.078	.039	039	.078	.078	.039	078	.078	<.039

oil plus beeswax Further, it is of particular interest to note that procaine penicillin in peanut oil gelled with 2% aluminum monostearate of a small particle size maintained a blood concentration over a much longer period than the same combination but with large particle sized penicillin No instances of undue irritation or untoward effects were reported by or observed in any of the subjects receiving the aluminum monostearate-peanut oil gelled products other than the occasional allergic reactions to penicillin The influence of particle size, per se, on maintenance of blood concentration will be reported later by Buckwalter, Dickison and this group

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PHARMACOLOGY OF PROCAINE PENICILLIN

By
Henry Welch and *Harold L. Hirsh**

Procaine penicillin in oil became available to clinicians February 10, 1948 Prior to the preparation of a under the Penicillin Regulations describing the standards identity, strength, quality and purity of this drug, a consid- amount of study had been devoted to establishing its safety efficacy These preliminary studies indicated that procaine cillin in oil would be one of the most important and valuable of the penicillin preparations for prolonging blood levels In the two months that have followed the advent of procaine penicillin, enormous quantities have been made available for use and, *in contrast* to the usual gradual acceptance of a new ration, the medical profession has rapidly adopted this of penicillin for therapeutic use In approximately two months, well over 3 5 trillion units of procaine penicillin in oil have been certified

Procaine penicillin is usually prepared by the double decom position of sodium penicillin and procaine hydrochloride The relatively insoluble salt resulting contains approximately 40 milligrams of procaine for each 60 milligrams of penicillin Thus, in the ordinary dose of 300,000 units, approximately 120 milligrams of procaine is injected Procaine is the least toxic of the more widely used local anesthetics, possessing approximately one fourth the toxicity of cocaine If the approximate fatal dose of cocaine for man is 1 2 grams, then the lethal dose of procaine would be on the order of 4 8 grams This would be equivalent to 12,000,000 units of procaine penicillin in a single injection However, as much as 3 0 grams of procaine have been employed in a single surgical procedure without untoward symptoms and, in addition, the insolubility of procaine penicillin and resulting slow release of procaine would have to be considered in arriving at an approximate fatal dose of the latter drug Furthermore, procaine is rapidly destroyed by the liver Animals are able to withstand repeated intravenous injections of large fractions of the minimum fatal dose and several times the average fatal intravenous dose can be administered to cats by slow infusion with survival of the animals Although no irritant or devitalizing action has been demonstrated for procaine, some

* Food and Drug Administration, Washington, D C

individuals are extremely sensitive to the central nervous system stimulant action of procaine In such cases, however, the onset is slow and any of the common barbiturates control the reaction Unfortunately, deaths have been reported following the administration of as little as 0 01 to 0 13 grams of procaine, and it is therefore important to determine the allergic state of an individual toward procaine before procaine penicillin is administered Although death in an idiosyncratic individual due to procaine has been an extremely rare occasion, consideration has to be given now to the fact that where thousands of patients are administered procaine as an anesthetic, tens of thousands will be injected with procaine penicillin

Prior to the acceptance of procaine penicillin in oil at least 2,000 individuals had received injections of the drug with no evidence of sensitization Since that time many thousands of injections have been made and to our knowledge there have been no authentic reports of sensitization It was expected that we would encounter not only the usual 3 6 per cent of patients sensitive to penicillin, but, in addition, those sensitive to procaine as well On the basis of data available to us at this time this is apparently not to be the case Lack of sensitization to procaine penicillin may be due to the slow release of procaine as the drug breaks down in the tissues bringing about subjective relief in a manner similar to that produced by benadryl and pyribenzamine Dreisbach and Nai Chu recently reported[1] that procaine failed to show protective effects against histamine toxicity, anaphylactic shock and the Arthus phenomenon, and alleviated some of the allergic symptoms by virtue of its analgesic effect, which prevented the pruritis usually associated with penicillin sensitivity

As was previously demonstrated[2,3] with other salts of penicillin, the toxicity of procaine penicillin is associated with the cation, the procaine portion of the molecule In mice the LD_{50} of procaine base was found to be approximately 1 06 milligrams, while that of procaine penicillin was found to be 2 45 milligrams On the basis that procaine penicillin contains approximately 40 per cent of the base the latter figure (2 45 mg) would appear to be within the limits of experimental error and that the toxicity observed is due to the procaine present

Similar results were obtained in both dogs and cats with intravenous or intramuscular injection Table 1 illustrates the relative effect of procaine penicillin and of procaine on a cat anesthetized with phenobarbital

Table I

PROCAINE PENICILLIN PHARMACOLOGY

EFFECT OF PROCAINE PENICILLIN AND OF PROCAINE ON BLOOD PRESSURE AND RESPIRATION IN THE CAT

CAT NO 284
WT 2.3 KILO
B.P 90mm/Hg
3-18-48

Injection	Substance	Dose	Respiration	B P Change
1	Proc Pen	12 mg/kg	Normal	+26 mm
2	Procaine	2.1 "	"	
3	"	4.2 "	"	− 6 mm
4	Proc Pen	24 "	Slowing	+ 15 "
5	Procaine	8.4 "	"	− 28 "
6	Proc Pen	36 "	Failure	Dead

RESP

BP

1 2 3 4 5 6

Doses of 2.0 and 4.0 mg./kg. of procaine exerted little effect while 8.4 mg./kg. resulted in a fall in blood pressure accompanied by a slowing rather than a quickening of respiration. Procaine penicillin in a dose of 12 mg./kg. did not affect the respiration but resulted in a blood pressure increase of 26 millimeters of mercury. Twenty-four mg./kg. caused a noticeable slowing in the respiration but the blood pressure increase was only about half that observed with the smaller dose. This dose corresponded approximately to the 8 4 mg./kg. dose of procaine which resulted in a slowing of respiration accompanied by a marked drop in blood pressure. The administration of 36 mg./kg. of procaine penicillin resulted in respiratory failure and death.

Table 2 illustrates comparative effects when graded doses of procaine and of procaine penicillin are administered to the phenobarbital anesthetized dog.

In each instance the blood pressure fall accompanying administration of procaine penicillin was more pronounced than the blood pressure fall accompanying injection of an equivalent dose of procaine alone. The recovery period was delayed with procaine penicillin in comparison with procaine alone.

When intramuscular injections of 300,000 units of procaine penicillin in oil were made in the cat there was no demonstrable effect on blood pressure or respiration. When an equivalent dose

TABLE 2
PROCAINE PENICILLIN PHARMACOLOGY
EFFECT OF PROCAINE PENICILLIN AND OF PROCAINE ON BLOOD PRESSURE AND RESPIRATION IN THE DOG

DOG B 15
WT 10 kg
BP 100mm
3 18 48

Injection	Substance	Dose	Respiration	B P Change mm/hg
1	Procaine	5mg/k	Slow	− 4
2	Proc Pen	12 "	Slow	− 20
3	Procaine	10 "	Slow	− 20
4	Proc Pen	24 "	Very Slow	− 38
5	Procaine	15 "	" "	− 26
6	Proc Pen	36 "	Rapid	− 38
7	Procaine	20	Very Rapid	Failure and Death

RESP

BP

1 2 3 4 5 6 7

of procaine in oil was injected, however, a slow, prolonged increase in blood pressure occurred and respiration became very shallow

After the injection of procaine penicillin in oil, urinary excretion of penicillin follows the same general trend that is obtained on injection of pencillin itself On the basis of the data obtained by two investigators, however, practically all, if not all, of the penicillin is recovered in the urine and for all practical purposes 100 per cent of the injected penicillin is recovered during the first 24 hours The results obtained on from 11 to 13 determinations at from six to 48 hours are shown in Table 3, where it will be noted that penicillin was still being excreted 48 hours after injection of 300,000 units intramuscularly, and furthermore that 97 per cent had been excreted during the first 24 hours The accumulative excretion figures in Table 3 indicate an excretion of greater than 100 per cent These results are probably associated with the inherent error in the biological assay procedure

The data upon which was based the claim that following the injection of 300 000 units of procaine penicillin in oil, blood concentrations of penicillin of 0 03 units or more would be obtained for 24 hours in at least 95 per cent of patients injected, were obtained from a study of 400 patients by 10

TABLE 3

PROCAINE-PENICILLIN

Average Ur nary Excret on of Pen c ll n Follow ng a S ngle I M Inject on of Proca ne pen c ll n in O l 300 000 Un ts Compos te Data—2 Invest gators

Hours after injection	Number of determ nations	Av vol ur ne ml.	Av U/ml	Cumulat ve excret on %
6	13	425	408	47
12	13	276	441	76
18	12	271	181	89
24	12	302	99	97
30	11	400	53	104
36	12	383	42	108
42	11	299	43	112
48	13	372	22	113

The results obtained following the intramuscular injection of 300 000 units of procaine penicillin in oil are shown in Table 4

It will be noted that in 49 patients an average blood level of 0 23 units per ml was obtained at the 24th hour in 96 per cent of the patients injected These results are typical of those obtained on preliminary trial of this drug However, apparently in the transition from pilot plant preparation of procaine penicillin to commercial production of procaine penicillin in oil some modification of the procaine penicillin took place It has been found that there is a variation in the ability of procaine penicillin in oil to produce blood levels at the 24th hour, not only from one manufacturer to another but within the lots produced by individual manufacturers A number of lots of procaine penicillin in oil from different manufacturers have been tested for

TABLE 4

PROCAINE-PENICILLIN

Average Pen c llin Blood Levels Follow ng Intramuscular Inject on of 300 000 U Proca ne Pen c ll n in O l

Hours after injection	No of determ nations	Av blood level U/ml	% w th 0 03u or more per ml
4	3	0 62	100
8	3	0 31	100
12	30	0 49	100
16	12	0 31	100
18	31	0 27	100
20	3	0 09	100
24	49	0 23	96
28	3	0 04	100

their ability to prolong blood concentrations of penicillin. Typical results obtained are shown in Table 5 where it will be noted that as few as 33 per cent of the patients injected show 24 hour blood levels following intramuscular injection of 300,000 units of procaine penicillin in oil with one lot, while with another lot injected similarly, blood concentrations of penicillin at the 24th hour were obtained in 100 per cent of the patients injected.

Studies have been made of the solubility of various manufacturers' procaine penicillin in both water and oil on the basis that the solubility in these two substances might have some *effect on the blood level following the intramuscular injection* into man. No significant differences in solubility in either water or oil in a study of 20 lots from 13 different manufacturers could be demonstrated. The solubility of procaine penicillin in oil appears to be insignificantly low while the solubility of procaine penicillin in water of the lots studied averaged 6500 units per ml. with very little variation from one lot to another. The relative density of procaine penicillin manufactured by nine different companies was determined and no significant differences demonstrated. Furthermore, the identity of procaine penicillin as exhibited by the refractive indices have been checked on procaine penicillin produced by 12 companies and the indices α1,685, B1,570 and γ1.540 were obtained in all instances. From the

TABLE 5

PROCAINE PENICILLIN

Typical Blood Penicillin Levels Following Injection of One Ml Containing 300,000 U Procaine Penicillin in Oil

Lot	Patient	Penicillin blood levels U/ml			% 24 hr level	% with 0.03u or more at 24hr
		16hr	20hr	24hr		
1	1	2.0	0.0	0.0		
	2	0.0	0.03	0.0		
	3	0.03	0.03	0.0		
	4	2.0	0.03	0.03		
	5	0.06	0.00	0.0		
	6	2.0	0.12	0.06	33	33
2	1	0.12	0.25	0.25		
	2	0.12	0.25	0.12		
	3	0.12	0.12	0.12		
	4	0.12	0.12	0.25		
	5	0.12	0.12	0.12		
	6	0.25	0.25	0.12		
	7	0.25	0.25	0.25	100	100
	8	0.25	0.12	0.25		

TABLE 6

PROCAINE-PENICILLIN

Typical Penicillin Blood Levels Following Intramuscular Injection of 300 000 u Procaine penicillin in Oil—Lot 3 Particle Size 81 2% Greater than 50 Microns

Patient no	Penicillin levels in blood 16hr	20hr	24hr	% 0 03u or above at 24hr
1	0 5	1 0	0 09	
2	0 5	0 25	0 12	
3	0 12	0 12	0 05	
4	0 5	0 25	0 25	
5	0 5	0 125	0 12	
6	0 25	0 25	0 25	
Average	0 4	0 33	0 14	100

TABLE 7

PROCAINE PENICILLIN

Blood Penicillin Levels with Procaine-penicill n in Oil 300 000 u I M Lot 4 Particle Size—74 7% 50 Microns or more

Laboratory	Patient no	U/ml 16hr	20hr	24hr	% 0 03u or more at 24hr
G	1	0 06	0 06	0 0	
	2	0 12	0 03	0 0	
	3	0 12	0 12	0 06	
	4	0 06	0 06	0 06	
	5	0 12	0 06	0 03	
	6	0 12	0 06	0 03	
	7	0 06	0 03	0 0	
	8	0 06	0 06	0 03	
	9	0 12	0 12	0 06	
	10	0 06	0 0	0 0	
	11	0 06	0 03	0 0	
	12	0 12	0 06	0 03	
	13	0 12	0 06	0 0	
	14	0 12	0 03	0 0	
	15	0 25	0 12	0 06	
	16	0 25	0 06	0 06	
Average		0 11	0 06	0 026	54%
F	1	2 0	0,12	0 06	
	2	0 5	0'12	0 12	
	3	2 0	0 12	0 03	
	4	2 0	0 25	0 12	
	5	0 5	0 06	0 25	
	6	0 5	0 00	0 00	
	7	2 0	0 00	0 00	
	8	2 0	0 12	0 06	
	9	2 0	0 25	0 00	
Average		1 5	0 11	0 07	66%

studies made it would appear that the procaine penicillin manufactured by the various producers of penicillin is identical

Examination of a large number of lots of procaine penicillin in oil has indicated that all would pass the requirements which were established for penicillin in oil and wax concerning particle size, namely that not less than 50 per cent of the total relative weight of the penicillin in the drug consists of penicillin having a particle size not less than 50 microns in length Certain lots of procaine penicillin in oil have been tested in the clinic to determine whether particle size of itself was an important factor in the ability of this preparation to prolong the blood levels of penicillin The results obtained on two lots, which are typical of the several tested, are shown in Tables 6 and 7

In Table 6 it will be noted that lot 3, on injection of 300,000 units, resulted in average blood levels at the 20th hour of over 0 3 unit and at the 24th hour of over 0 1 unit per ml, and that 100 per cent of the patients tested showed 0 03 unit or more at the 24th hour The particle size of this lot was 81 2 per cent based on the specification originally adopted for penicillin in oil and wax. In contrast to this, examination of lot 4 (Table 7) by two laboratories resulted in blood concentrations of penicillin of 0 03 unit or more at the 24th hour in 54 per cent and 66 per cent, respectively, of the patients injected with 300,000 units The particle size of lot 4, however, was 74 7 per cent, based on the penicillin in oil and wax specifications, and this, on the basis of the error inherent in this test, is not significantly different from the results obtained with lot 3 (Table 6)

In Table 8 are summarized data from seven different investigators

TABLE 8

PROCAINE-PENICILLIN

Average Blood Levels — Composite Data From 7 Investigators

Hours after injection	Number of determination	Av U/ml serum	% showing 0 03 U/ml or greater
6	35	0 47	100
12	193	0 25	99 5
16	39	0 19	95
18	88	0 21	93 2
20	64	0 13	67 2
24	246	0 15	77 7
30	53	0 07	47 2
36	86	0 06	36 1
48	104	0 02	27

Each of the patients in this group was injected with 300,000 units of procaine penicillin in oil and blood determinations for penicillin made from the sixth to the 48th hour It will be noted that approximately 78 per cent of the patients so injected had blood levels of 0 03 unit per ml or more at the 24th hour Approximately 250 determinations were made at this hour It will be noted also that in this table the marked change in blood level concentrations occurred between the 18th and 20th hours, since at this time the per cent showing blood concentrations of penicillin of 0 03 or more were 93 per cent at the 18th hour and only 67 per cent at the 20th hour

Discussion

The evidence available on procaine penicillin indicates that it is one of the most important developments made by the drug industry since penicillin was first produced in this country In addition to the fact that procaine penicillin prolongs blood concentrations of penicillin longer and at higher levels than any other previously developed product, this drug has several other advantages On injection into the muscle, procaine penicillin appears to be less painful than the injection of aqueous solutions of sodium, potassium or calcium penicillin This lack of pain is probably due to the slow release of procaine which results in a local anesthetic effect In addition, the evidence available indicates that we will obtain a marked decrease in the number of individuals sensitive to this drug over those normally found sensitive to penicillin Lack of sensitivity following injection of procaine penicillin is probably due to the procaine in the procaine penicillin molecule, but the mode of its action is not clear at the moment Dreisbach and Nai Chu have reported recently that procaine is not active as an anti allergic agent in certain forms of allergy, such as anaphylactic shock and the Arthus phenomenon and that procaine is not successful in neutralizing the effect of histamine in guinea pigs These authors however, state that procaine does act in a subjective manner similar to pyribenzamine and benadryl in certain allergic states In any case as a precautionary measure, individuals injected with procaine penicillin should first be tested for their sensitivity to procaine, since in certain rare instances individuals sensitive to procaine may react violently to injections of this drug Fortunately, the insoluble nature of procaine penicillin reduces the possibility of reaction to procaine, since its slow release and rapid destruction by the liver mitigates against the

possibility of violent reactions following injection of this material Those individuals who are extremely sensitive to the stimulant action of procaine on the central nervous system are readily controlled by the common barbiturates Consistent with the prolonged blood concentrations of penicillin produced following injection of procaine penicillin is the prolonged excretion of penicillin in the urine Following the deep intramuscular injection of 300,000 units of procaine penicillin in oil, penicillin may be recovered in the urine from two to four days Furthermore, it would appear that practically all of the penicillin injected is recoverable in the urine The toxicity that may be demonstrated in animals for penicillin is associated with the procaine portion of the molecule and this may be demonstrated by intravenous injection of mice, rabbits, cats and dogs

Unfortunately, recent studies have indicated that the preliminary outstanding results obtained with procaine penicillin in oil have proven not to be consistent in all lots of procaine penicillin in oil produced The evidence is that certain lots of procaine penicillin in oil fail to prolong blood concentrations of penicillin to the extent originally found on the preliminary batches of this product This discrepancy cannot be associated with the density, solubility in water and oil, or the crystallographic identity of the procaine penicillin molecule The particle size of the procaine penicillin utilized in the preparaton of procaine penicillin in oil does not appear to be of the same significance as the particle size of the penicillin utilized in the preparation of penicillin in oil and wax This might be expected, in view of the fact that these two preparations depend on entirely different phenomena for their efficacy in prolonging blood concentrations of penicillin In the case of penicillin in oil and wax prolongation of blood concentrations of penicillin is dependent upon a protective effect of the oil and beeswax utilized as a menstruum in this drug Protection of the penicillin particles in the oil and wax mixture is probably due to the hydrophobic action of the oil and wax In the case of procaine penicillin in oil, prolongation of blood concentrations of penicillin is associated mainly with the insolubility of the procane penicillin particles and the influence of the hydrophobic nature of the oil is probably minor in character It may be theorized, since prolongation of blood concentrations of penicillin is dependent upon the insoluble characteristics of procaine penicillin, that the smaller the particle of procaine penicillin, the more rapidly it would be absorbed, since greater surface areas are

exposed to tissue fluids for the smaller particle size If this is true, it would follow that micro crystalline procaine penicillin would be relatively rapidly absorbed from an oil menstruum and prolongation of blood concentrations of penicillin would not be obtained Some preliminary evidence along this line has recently been obtained by one of the manufacturers* who has shown that when a lot of procaine penicillin in oil contains a relatively large amount of "procaine penicillin dust," such lots fail to prolong blood concentrations of penicillin materially but do show relatively high blood concentrations of penicillin in the early hours following intramuscular injection Studies in our laboratory have indicated that lots of penicillin which do not meet the standards for prolongation of blood concentrations of penicillin originally set by this product are in the minority Intensive investigations will be continued with a view toward obtaining the facts which will make it possible to assure clinicians of a uniform and efficient product

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* Chas. Pfizer and Co , Inc personal communication Dr R A Patelski

THE TREATMENT OF EARLY SYPHILIS WITH PROCAINE PENICILLIN G IN OIL

By

Harold L Hirsh, S Ross Taggart, and *Henry Welch**

As part of the study to evaluate the efficacy of procaine penicillin G in oil the treatment of early syphilis with this preparation was undertaken This publication is a report of the preliminary results

The dose of procaine penicillin G in oil selected was 600,000 units (2 cc) intramuscularly daily for 5 days for a total of 3 000 000 units Included in the study were 54 patients, 3 with seropositive darkfield positive primary lesions, 20 with seronegative darkfield positive primary lesions, 27 with secondary syphilis, and 4 with early latent syphilis These patients have now been followed for a period of one to three months

The therapeutic results were evaluated on the basis of the following criteria (1) disappearance of *Treponema pallida* from lesions, (2) healing of lesions, (3) changes in serology Six patients with chancres, who were examined 24 hours after the initial dose of 600,000 units of procaine penicillin G in oil, showed a complete disappearance of the spirochetes from the lesions at that time All the patients with primary or secondary [illegible] the period of time during [illegible] lowing the administration [illegible] ronegative chancres, none has developed positive serologies during the follow up period All of the patients with positive serologies have shown progressive decrease in the serological titers

The patients were followed carefully for the development of Jarisch-Herxheimer reactions Only two patients in the series had findings which were suggestive of this type of reaction

None of the patients has had a local reaction or evidences of hypersensitivity

A larger series of patients followed for a longer period of time will be necessary before the value of this penicillin preparation can be adequately ascertained These preliminary results appear encouraging

* From the Georgetown University School of Medicine the Bureau of Venereal Diseases District of Columbia Health Department and the Food and Drug Administration Federal Security Agency Washington, D C

THE EFFECT OF HUMAN CEREBROSPINAL FLUID ON THE DILUTION BIOASSAY OF PENICILLINS G, X AND K*

By
Harold A. Tucker

Numerous workers have been unable to detect penicillin in the cerebrospinal fluid of man after extrathecal administration of the drug in the usual therapeutic doses Significant concentrations may, however, be demonstrated with some degree of regularity in the presence of some forms of bacterial meningeal irritation[1], or when massive intravenous dosages are given[2] (See Dumoff-Stanley and her coworkers[3], Schwemlein and others[4], for detailed discussion) It is the purpose of this preliminary report to point out that human cerebrospinal fluid exerts an inhibitory effect on the bactericidal activities of penicillins G, X and K *in vitro* as determined by a serial dilution bioassay method This effect is comparable in some ways to that exerted by human serum[5,6], but differs in several important respects Whatever the mechanism involved, the inability to demonstrate even traces of penicillin in normal cerebrospinal fluid after its administration in average therapeutic dosage may be related to the inhibitory effect of spinal fluid on the bactericidal activity of the drug

METHOD AND MATERIAL

Individual and pooled cerebrospinal fluid specimens obtained from normal and syphilitic patients were used in this study The syphilitic patients were either untreated, or had previously received penicillin therapy for neurosyphilis, but in no case more recently than 3 weeks previously Total protein determinations were carried out by a turbidimetric method (Klett-Summerson) on several pooled specimens, and in no case did this value exceed 50 milligrams per cent

The principle of the method here employed has been described in a similar study on individual and pooled human sera[6]. In the control assay, containing no cerebrospinal fluid, varying amounts of a penicillin dilution in broth (0 8, 0 72, 0 6, 0 48, 0 4, etc), were adjusted to a total volume of 0 8 cc with broth A 4 per cent broth suspension of defibrinated human group O blood was

* ... United ... raduate ... in the ... Eagle, Director

inoculated with the C-203 strain of *Streptococcus pyogenes*, 0 5 cc was then added to each tube to serve as the hemolytic indicator of growth The endpoint was the tube containing the smallest amount of penicillin which had completely inhibited hemolysis after 6 hours' incubation at 37° C followed by 10 to 12 hours at room temperature

In order to determine the effect of cerebrospinal fluid on the results of this assay, dilutions of penicillin in 96, 48, 24, 12 and 6 per cent spinal fluid were similarly distributed, and the volume adjusted to 0 8 cc with the corresponding spinal fluid-broth diluent (e g, one volume of 1 1,000,000 penicillin in broth was diluted with 24 volumes of 50 per cent spinal fluid to give a 1 25,000,000 dilution in 48 per cent cerebrospinal fluid This was distributed and the volumes adjusted to 0 8 cc by the addition of 48 per cent spinal fluid in broth) In this manner a constant percentage of cerebrospinal fluid was maintained in each tube of the assay Finally, as with the control assay, 0 5 cc of red blood cell suspension was added to each tube, this reduced the concentration of spinal fluid by 5/13 (0.8/0 8 + 0 5) Thus an initial concentration of 96 per cent fell to 59 per cent, 48 per cent to 29 5, etc, as indicated in Tables I and II The amount of penicillin necesary to inhibit hemolysis in the presence of varying concentrations of spinal fluid, compared to the amount necessary in its absence, provided a direct measure of the degree to which the activity of the drug had been inhibited by a particular concentration of cerebrospinal fluid

Results

The results of 11 determinations with each penicillin studied (G, X and K) are presented in Table I Significant differences were not found between individual and pooled specimens nor did fluids obtained from normal subjects differ demonstrably in their inhibitory effect from those taken from patients with untreated or malaria and/or penicillin treated neurosyphilis The results in the 2 groups were therefore combined and the resulting average curves are shown in Figure I

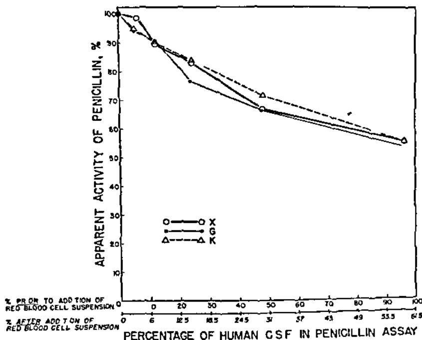
Penicillins G, X and K proved identical in their susceptibility to the inhibitory effect of *spinal fluid* Further, the inhibitory effect resembled that of human serum on penicillins G and X The effect of unheated human serum on penicillin K, however, [illegible] than that exerted by cerebrospinal fluid Thus, the apparent activity rasted to 36 per cent

Inhibitory Effect of Human Cerebrospinal Fluid on the In Vitro Activities of Penicillin G, X and K

Penicillin Species	Percentage of Spinal Fluid In Assay		Relative Activity of Penicillin In Spinal Fluid Specimen Number, (Referred to its Activity in Broth Control as 100)											Relative Activity	
	Before addition of red blood cell suspension	After addition of red blood cell suspension	1	2	3	4	5	6	7	8	9	10	11	Mean	Median
G	96	59	56	56	50	50*	60	55	55	50	55	55	45	53 4	55 0
	48	29 5	75	75	67	60	73	67	61	61	67	61	60	66 1	67 0
	24	14 8	75	75	83 5	75	86	79	73	67	76	76	75	76 5	75 0
	12	7 4	100	90	83 5	90	100	92	92	83	100	92	75	90 5	92 0
	6	3 7	100	100	83 5	95	100	92	100	91	100	92	90	94 0	95 0
X	96	59	56	60	50	50	50	50	61	55 5	55 5	62	60	55 5	55 5
	48	29 5	60	69	60	55 5	55 5	55 5	71 5	71 5	71 5	80	80	66 4	69 0
	24	14 8	90	100	80	80	80	73	83 5	83 5	83 5	80	80	83 0	80 0
	12	7 4	100	100	80	86	86	80	100	100	91	80	80	89 4	86 0
	6	3 7	100	100	86	100	100	100	100	100	100	100	100	98 7	100 0
K	96	59	53	53	62 5*	62 5*	62 5*	62 5*	54 5	50	54 5	46*	46*	55 2	54 5
	48	29 5	68	63	91	83	83	83	63	75	63	61	52 5	71 4	68 0
	24	14 8	79	——	100	100	100	83	75	75	75	73 5	73 5	83 4	77 0
	12	7 4	95	——	100	100	100	100	80	80	80	92	78	90 5	93 5
	6	3 7	100	100	100	100	100	100	80	80	80	100	100	94 5	100 0

* Determinations with spinal fluid from nonsyphilitic subjects

FIGURE I



INHIBITORY EFFECT OF HUMAN CEREBROSPINAL FLUID ON THE IN VITRO ACTIVITIES OF PENICILLINS G X AND K

The abscissas are the percentages of spinal fluid present in the penicillin assay before and after the addition of the red blood cell suspension (hemolytic indicator) The ordinates are the mean apparent activities of the penicillins

for G and 40 per cent for penicillin X, while in the presence of 59 per cent spinal fluid, the values for the activities of the 3 penicillins were 55, 53, and 56 per cent respectively

Preliminary studies (not cited in the tables) have shown that this inhibitory effect was not diminished appreciably by heating at 100° C for 30 minutes, followed by Seitz filtration of the spinal fluid Changes in hydrogen ion concentration within the range of pH 7 3 to 8 4, did not alter the effect As will be described in a following paper, when various concentrations of the penicillins were incubated in 96 per cent cerebrospinal fluid [illegible] a loss of penicillin activity and

TABLE II

Effect of Human Cerebrospinal Fluid on the Bioassay of Penicill ns G, X and K Interpolated corrective factors

(After Table I Figure I)

Amount of spinal fluid (in total volume of 0 8 cc) in indicator tube of assay *	Percentage of spinal fluid		Corrective factors (means) for pen cill ns G X and K **
	before addit ion of red cell suspens on	after addit ion of red cell suspen sion	
0 8	whole fluid =100	61 5	1 8
0 7	1 1 1 =87 5	54	1 7
0 6	1 1 3 =75	46	1 65
0 5	1 1 6 =62 5	38 5	1 6
0 4	1 2 =50	31	1 5
0 3	1 2 5 =37 5	23	1 4
0 2	1 3 =25	15 5	1 3
0 15	1 4 =19	11 5	1 2
0 10	1 8 =10	6	1 1
< 0 10	1 16+ < =7	< 5	1 0

* Smallest amount of penicillin which in a total volume of 0 8 cc inh b ted completely hemolys s of human red cells by the C-203 strain of *Strep pyogenes*

** Factors by which the apparent concentration of penicillin in a particular con centrat on of spinal fluid must be multiplied to compensate for the inhib tory effect of the fluid on the assay

Discussion

Although the inhibitory effect of human serum on the bioassay of penicillin has been ascribed, at least in large part, to the binding effect of the plasma proteins (particularly of the albumin fraction)[7], such a mechanism does not explain the similar property of spinal fluid For penicillins G and X, the inhibitory effect of cerebrospinal fluid was only 15 to 20 per cent less than that of serum while the total protein contents differed by a factor of 100-to 300-fold Further, heat-coagulation of the protein and its removal by means of Seitz filtration, did not modify appreciably the inhibitory effect Finally, the fact that penicillin, incubated in cerebrospinal fluid, progressively lost activity, suggested that there might be actual *destruction* of penicillin by some as yet unidentified mechanism similar to that observed in the case of serum[5] Further studies are in progress and will be reported subsequently

From the practical standpoint, it becomes feasible to correct the results of bioassays on human cerebrospinal fluid for the

inhibitory effect exerted by the fluid, provided only that we know the concentration of spinal fluid in the indicator tube of the assay In Table II are given corrective factors by which the apparent penicillin content should be multiplied in order to compensate for this inhibitory effect Since the curves for penicillin G, X and K did not differ significantly (Figure I), the same corrective factors apply to all three species The corrections are significant only when the final concentration of spinal fluid (after the addition of the red blood cell suspension), exceeds 7 8 per cent These findings are of interest primarily to the investigator concerned with the pharmacology and mode of action of penicillin For the physician, the uncorrected concentrations may be more useful since such values probably more nearly represent the bactericidal activity of a given penicillin

The experimental data here reported may explain, at least in part, the discrepancy between the apparent failure of penicillin to penetrate into the cerebrospinal fluid in man, and the profound therapeutic response frequently obtained in, for example, neurosyphilis They suggest that effective concentrations of penicillin may well be present, but not demonstrable by current *in vitro* methods of bioassay because of the inhibitory effect of cerebrospinal fluid itself on the assay

Summary

Human cerebrospinal fluid exerts a hitherto undemonstrated inhibitory effect on the bactericidal activities of penicillins G, X and K *in vitro*, as determined by a serial dilution bioassay method

This effect is the same for the 3 species of penicillin studied, and is comparable in magnitude with that exerted by human serum on penicillin G and X (not penicillin K) Preliminary evidence suggests that protein binding is not the sole or even an important, mechanism involved, but that there may be actual destruction of penicillin by the cerebrospinal fluid

Corrective factors are tabulated which compensate for the inhibitory effect of human cerebrospinal fluid on the *in vitro* bioassay of penicillins G, X and K.

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ORAL ADMINISTRATION OF BACITRACIN

By
Glenn C Bond, Milton J VanderBrook, Jean L Wiley,
and *Mary Ann Nook**

Bacitracin, as defined by the Food and Drug Administration is the antibiotic substance or substances produced by the growth of the "Tracy I" strain of Bacillus subtilis This material is now being produced on a pilot scale and has been made available for study by a few commercial firms Several reports have been published regarding the efficacy of this antibiotic, when administered topically or systemically, but information concerning oral use of the antibiotic is very meager

The purpose of this paper is to present a preliminary report of the findings following oral administration of bacitracin to dogs Results will concern the effect of bacitracin on the intestinal flora, and bacitracin levels in the feces, blood and urine

Scudi, Clift and Krueger in the Proceedings of the Society for Experimental Biology and Medicine, May 1947 report that following the oral administration in dogs of 3,000 u and 6 000 u of bacitracin per kg body weight, no detectable bacitracin was found in the blood stream, nor was any found in the urine during the 24 hours after administration of the antibiotic Following oral administration of 1,500 u per kg body weight to 2 dogs, less than 5% of the oral dose was recovered in the stool From these findings they concluded that the antibotic was largely destroyed in the intestinal tract

Dr Frank Meleney has reported the use of oral bacitracin in conjunction with systemic administration, in the treatment of a case of colitis Clinically good results were obtained

* Research Laboratories The Upjohn Company, Kalamazoo, Michigan.

Six dogs have been studied by the following procedures All dogs were put on a uniform diet several days preceding and during the experiment Bacitracin in solution was administered by stomach tube twice daily, at 8 30 A M and 4 30 P M The amounts given varied from 2,000 u /kg body weight per day to 20,000 u /kg body weight per day Heparinized blood samples were taken at 1 and 2 hours, after administration of the morning dose of bacitracin Fecal specimens were obtained by means of a fecal hook each morning before the administration of bacitracin and in 4 of the 6 dogs 24 hour urine samples were collected The dogs exhibited no gross change in activity Body weight was maintained and appetite as measured by food consumption was not affected

To study the effect of bacitracin on the intestinal flora, a procedure was used similar to that used by Poth for the study of the effect of sulfonamides and streptomycin on the intestinal flora of dogs

A portion of each specimen of feces was diluted 1-10 with 5% $K SO_3$ We have shown that in optimum proportions bacitracin is inactivated by K_2SO_3 After standing for 30 minutes at room temperature tenfold serial dilutions in saline were carried through 10^{10}, and samples from these dilutions were seeded into the test media

To determine the relative percentages of Gram + and Gram — organisms, fluid thioglycollate medium was inoculated and incubated 48 hours at 37° C Smears were made and examined to determine the highest dilution at which Gram positive and Gram negative organisms were found

The presence of fecal streptococci was determined by inoculating tubes of sodium azide medium, incubating at 37° C for 48 hours Direct smears were made from all tubes showing growth and examined for the presence of streptococci

Anaerobic agar was inoculated and heated to 80° C for 15 minutes, incubated 37° C for 48 hours to determine the presence of spore forming anaerobes

To determine the number of coliform organisms, poured plates of eosin methylene blue agar were prepared

Fecal, blood and urine specimens were assayed for bacitracin by the method described by Bond and Nook in *Science*, Feb 27, 1948 A hemolytic streptococcus was the test organism and the standard bacitracin is that supplied by the Food and Drug Administration

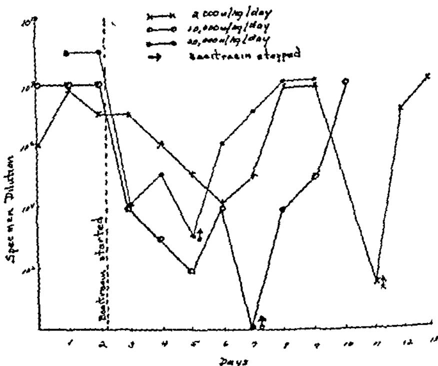


Fig. 1 Effect of Bacitracin on Fecal Streptococci

The effect of bacitracin on the intestinal flora of dogs may be seen in the following figures and tables:

Fig. 1 shows the effect of bacitracin on fecal streptococci At the dosage level of 2,000 units per kg. per day, there is a marked variation in total count with the lowest count on the last day of treatment At a dosage of 10,000 u /kg per day the count drops abruptly and goes to zero on the 5th day of treatment When treatment is stopped there is a rapid rise, so that normal levels are reached in 3 days The 20,000 u treatment curve is similar to the 10,000 u except that treatment was stopped on the third day which was too soon to allow the count to drop to zero.

Fig 2 shows the effect of bacitracin on the spore forming anaerobes The results are comparable to those found for the fecal streptococci A 2,000 u dose decreased the count but the level does not remain constant during the course of treatment Both the 10,000 u dose and the 20,000 unit dose produce an immediate and rapid drop to zero and remain there until cessation of treat-

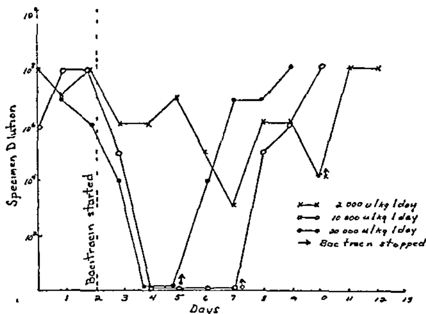


Fig 2 Effect of Bacitracin on Spore Forming Anaerobes

ment At that time there is a rapid and sharp rise to normal levels within 2 to 3 days

Fig 3 expresses graphically the relative percentage of Gram-positive and Gram negative organisms with a dose of 10,000 u /kg per day The pre-treatment samples show a greater incidence of Gram-positive organisms, but within 24 hours after treatment is started there is a shift so that the Gram negative organisms predominate during the course of treatment 24 hours after cessation of treatment the ratio is back to the normal relationship The figures in the graph are highest dilutions at which the organisms are found

No significant change was noted in the coliform organisms

Table 1 shows the amount of bacitracin per gram of feces At the 2,000 u dose, the feces of one dog contained measurable amounts of bacitracin in 3 of 4 days and for 2 days after cessation of treatment The other dog on the 2,000 u did not show measurable levels until the last day of treatment and the first day after treatment had stopped With 10,000 u both dogs showed definite levels of bacitracin persisting 1 to 3 days after cessation of treatment W[illegible]

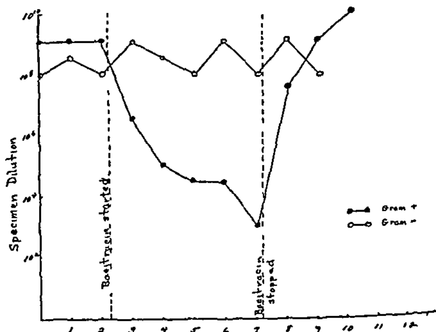


Fig 3. Effect of Bacitracin (10,000 u/kg/day) on Gram + and Gram − Organisms

bacitracin for the 3 days of treatment and persisting for 2 days after treatment It was not possible to determine the total bacitracin in the feces.

Table 2 shows the blood levels expressed as units of bacitracin per cc of plasma which were obtained following the oral administration of bacitracin. 2,000 u /kg. per day is not sufficient

TABLE I

Fecal Level of Bacitracin Following Oral Administration Expressed as Units of Bacitracin per Gram of Feces

Dose u/kg/day	Pre Treatment	Treatment Period Day 1	2	3	4	5	Post Treatment Day 1	2	3
2 000	0	09	56	0	6		1 19	6	0
2 000	0	0	0	0	0	1	4	0	
10 000	0	1 5	1 9	2 5	3 0		3 4	2 7	6
10,000	0	1 0	12 0	4 2	8 7		4	0	
20 000	0	75 0	75 0	15 0			7 0	8 0	0
20 000	0	90 0	85 0	100 0			18 0	9 0	0

TABLE 2

Blood Levels of Bacitracin Following Oral Administration   Expressed as Units per cc of Plasma

Dose u/kg/day	Pre Treatment	Treatment Period								Post Treatment	
		Day 1		Day 2		Day 3		Day 4			
		1 Hour	2 Hours	1 Hour	2 Hours	1 Hour	2 Hours	1 Hour	2 Hours	Day 1	Day 2
2 000 u	0	0	0	0	0	0	0	0	0	0	0
10 000 u	0	0		013		048		045		0	0
10 000 u	0	085	08	064	045	046	0	058	047	0	0
20 000 u	0	1	095	088	07	22	1			0	0
20 000 u	0	11	098	12	08	55	17			0	0

TABLE 3

Urine Levels of Bac tracin Following Oral Adm nistration

Dose u/kg/day	Pre Treatment	Treatment Period								Post Treatment					
		Day 1		Day 2		Day 3		Day 4		Day 1		Day 2		Day 3	
		u/cc	Total u	u/cc	Total u	u/cc	Total u	u/cc	Total u	u/cc	Total u	u/cc	Total u	u/cc	Total u
2 000	0	0	0	045	10 5	0	0	0	0	046	11 9	0	0	0	0
10 000	0	06	8 21	22	49 5	096		03	6 75	1	9 0	04	12 7	025	4 75
20 000	0	55	2550	8	2080	18	15 0			075	15 0	054	17 8	0	0
20 000	0	9	3690	5	1550	1 65	792			17	73	0	0	0	0

dosage to produce detectable blood levels 10,000 u /kg per day produced levels of similar magnitude in 2 doys 1 hour after administration The 2 hour levels in all cases are lower than the 1 hour level and in one 2 hour sample no bacitracin could be demonstrated No levels were found 24 hours after cessation of treatment With 20,000 u /kg per day the plasma levels were higher in all cases than with 10,000 units Again the 2 hour levels were lower than the 1 hour and no bacitracin could be demonstrated 24 hours after cessation of treatment

In Table 3, urine levels of bacitracin are given for 4 of the 6 dogs studied The urine was collected at 24 hour intervals and assayed 2,000 units daily gave very low and irregular results With 10,000 units a definite urinary level was found for each day of treatment and for 3 days following treatment 0 22 units per cc of urine was the highest level obtained at this dosage The two dogs on the 20,000 unit dose showed high urinary levels for the 3 days of treatment and in one case detectable levels persisted for 2 days post treatment The highest level obtained was 1 65 u /cc of urine

From the small series of animals receiving oral bacitracin, the following significant facts were obtained.

1 Fecal streptococci and spore forming anaerobes are greatly reduced in the intestine at a dosage between 2,000 and 10,000 u /kg per day

2 Coliform organisms are little affected by bacitracin

3 Bacitracin persists in the feces as long as 3 days following cessation of treatment

4 Bacitracin is absorbed from the intestinal tract as evidenced by significant blood levels 10,000 u /kg per day is sufficient to produce blood levels as high as 085 u /cc of plasma 1 hour after administration

5 Additional evidence of absorption was obtained by the finding of high levels of bacitracin in the urine The highest obtained with 10,000 units was 22 u /cc and with 20,000 units 1 65 u /cc of urine

THERAPEUTIC ACTIVITY OF BACITRACIN IN RABBIT SYPHILIS, AND ITS SYNERGISTIC ACTION WITH PENICILLIN A PRELIMINARY REPORT*

By
Harry Eagle, and *Ralph Fleischman***

Bacitracin is an antibiotic present in culture filtrates of *B subtilis*[1] In a previous report from this laboratory[2] it was shown to be actively treponemicidal in vitro against the non pathogenic Reiter strain Concentrations of 0 004 units per cc prevented growth, and 0 1 unit per cc killed 99 9 per cent of the organisms within 48 hours Against the pathogenic *T pallidum* in vivo, doses as small as 72 units/kg given intramuscularly caused the temporary disappearance of the organisms from rabbit testicular chancres[2] (It may be noted parenthetically that this dosage provides plasma levels comparable to those effective against the non pathogenic Reiter strain in vitro in both rabbits and man the plasma concentration remains in excess of 0 1 unit per cc for approximately 2 to 3 hours, and in excess of 0 01 unit per cc for approximately 6 to 8 hours[3]) At this small dosage however, the organisms regularly reappeared in the lesions days weeks or even months after the injection, and it required single doses of 5000 units/kg and more to effect the permanent healing of the primary lesion

The present paper is a preliminary report on the therapeutic activity of bacitracin in rabbit syphilis when aqueous solutions were injected intramuscularly once daily for four days Further, data are given which suggest a remarkable synergistic action between penicillin and bacitracin The relatively small numbers of animals make the results of qualitative rather than quantitative significance The synergistic action was nevertheless striking, and the incomplete data are reported at this time because they indicate the desirability of a clinical trial of bacitracin and penicillin used in combination in the treatment of human syphilis

METHODS AND MATERIALS The method of evaluating cure in the treated rabbits by lymph node transfers to normal animals

* This paper is taken almost verbatim from a paper now in press. (Proc. Soc. Exp Biol and Med)

** From the Laboratory of Experimental Therapeutics of The U S. Public Health Service and The Johns Hopkins School of Hygiene and Public Health Baltimore Maryland

4 to 6 months after treatment has been adequately described in previous reports from this laboratory[4,5]

The bacitracin used was obtained from the Ben Venue Laboratories, Bedford, Ohio, and the various lots assayed at 18 to 41 units per mg , while the penicillin was a commercial preparation of crystalline Penicillin G, generously supplied by the Commercial Solvents Company (Lot No 46042605, assaying at 1500 units/mg) The dosages of penicillin indicated in Fig 1 and Table I were adjusted for 1667 units/mg (1 11 mg administered instead of 1, etc)

Aqueous solutions of the two drugs were injected into the muscles of the thigh, on opposite legs, within a minute of each other, and the injections were repeated once daily for 4 days

EXPERIMENTAL RESULTS Table I lists the proportion of animals cured by various dosages of bacitracin, used a) either alone or in combination with total dosages, or b) 1, c) 4, or d) 16 mg /kg of penicillin G The dosage of penicillin G which used alone cured 50 per cent of the animals on this schedule of treatment had previously been shown to be on the order of (approximately) 40 mg /kg[5] The last 2 vertical columns of the table give the CD_{50} dosages of bacitracin, calculated by the Miller Tainter method[6] The standard error of those CD_{50} values is of necessity large because of the small number of animals, the trend is nevertheless clear On this schedule of treatment, approximately 9200 units/kg of bacitracin was necessary to cure 50 per cent of the animals (CD_{50}) When as little as 1 mg /kg of penicillin was simultaneously administered the curative dose of bacitracin was reduced from 9200 ± 2200 to 1280 ± 310 units/kg In other words approximately 1/40th of the CD_{50} dose of penicillin and 1/7th of the CD_{50} dose of bacitracin were curative when used in conjunction Further increase in the dosage of penicillin, from 1 to 4 to 16 mg /kg caused a progressive decrease in the dosage of bacitracin from 1280 to 840 to 480 units/kg In Fig 1, the top dotted line represents what the curative dose of bacitracin would have been were bacitracin and penicillin merely additive in their therapeutic action

... experimental data devi-
synergistic
bacitracin,
, markedly
reduced the amount of the second drug necessary for cure

THERAPEUTIC ACTIVITY OF BACITRACIN IN RABBIT SYPHILIS, AND ITS SYNERGISTIC ACTION WITH PENICILLIN. A PRELIMINARY REPORT*

By

Harry Eagle, and *Ralph Fleischman***

Bacitracin is an antibiotic present in culture filtrates of *B. subtilis*[1]. In a previous report from this laboratory[2] it was shown to be actively treponemicidal in vitro against the non-pathogenic Reiter strain. Concentrations of 0.004 units per cc. prevented growth, and 0.1 unit per cc. killed 99.9 per cent of the organisms within 48 hours. Against the pathogenic *T. pallidum* in vivo, doses as sm [illegible] caused the temporary [illegible] chancres[2]. [illegible] provides plasma levels comparable to those effective against the non-pathogenic Reiter strain in vitro: in both rabbits and man the plasma concentration remains in excess of 0.1 unit per cc. for approximately 2 to 3 hours, and in excess of 0.01 unit per cc. for approximately 6 to 8 hours[3].) At this small dosage, however, the organisms regularly reappeared in the lesions days, weeks or even months after the injection; and it required single doses of 5000 units/kg. and more to effect the permanent healing of the primary lesion.

The present paper is a preliminary report on the therapeutic activity of bacitracin in rabbit syphilis when aqueous solutions were injected intramuscularly once daily for four days. Further, data are given which suggest a remarkable synergistic action between penicillin and bacitracin. The relatively small numbers of animals make the results of qualitative rather than quantitative significance. The synergistic action was nevertheless striking; and the incomplete data are reported at this time because they indicate the desirability of a clinical trial of bacitracin and penicillin used in combination in the treatment of human syphilis.

METHODS AND MATERIALS. The method of evaluating cure in the treated rabbits by lymph node transfers to normal animals

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** From the Laboratory of Experimental Therapeutics of The U. S Public Health Service and The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.

HERXHEIMER REACTIONS IN PENICILLIN TREATMENT OF SYPHILIS IN PREGNANCY

By
Jack H Bowen, H N Cole, J R Driver, Richard C Light, and *John R Rauschkolb,*

in collaboration with M H Gustafson, Burt Held, J. M Kam, Manly Utterback, and A E Walker (Department of Dermatology and Syphilology, Western Reserve University)

From October 1943 to early 1947, using varying dosage forms of penicillin, there have been four cases of congenital syphilis among 151 active cases of syphilis in pregnancy Two unquestionable cases of fatal foetal or placental Herxheimer reactions occurred It is believed in acute syphilis complicating pregnancy, that under these conditions there should be either a few days preliminary treatment with bismuth or small doses of an arsenical or of penicillin in low dosage before regular penicillin therapy is instituted

Such a large number of the mothers relapsed after delivery, (forty four), that it is obligatory to have a monthly physical and serologic check up until such a time as they have been completely normal for one year

THE TIME COURSE OF THERAPEUTIC IMPROVEMENT IN NEUROSYPHILIS

By
George D Gammon and *John H Stokes*
(Penicillin Panel, the University of Pennsylvania)

An analysis of the time at which abnormal spinal fluids revert to normality has been made on a portion of our case material which includes the various types of neurosyphilis except paresis and taboparesis One group of 142 treated May, 1945 to October, 1946 was given a unit dosage of 4 8 million units of mixed penicillin administered in three ways 4 8 in 8 days, 4 8 in 16 days, and 2 4 in 8 days repeated in 4 months The other group of 72 was given G penicillin 9 6 million units (a few 4.8 million)

When estimated as cumulative percentage reaching normal, the two 4 8 groups move along identical curves to reach 70 and

72%; the 2 4 repeated group to 52% The curve for the latter is consistently lower throughout the 2½ years The curve of the G group appears to be following that of the 2 4 group, only about 35% have become normal

BIOASSAY OF PENICILLIN BY THE MOUSE-RABBIT TECHNIQUE

By

Paul D Rosahn, Boris Gueft, and *Catherine L Rose*
(Department of Pathology, New Britain General Hospital)

Ten days following the intraperitonal inoculation of mice with rabbit syphiloma material, they were treated by intramuscular injections of penicillin, the total calculated dose being divided into eight equal daily injections In the first experiment, sodium penicillin was assayed Groups of mice received from 4,000 to 64,000 units per kilogram of body weight, the treatment having been begun 45 days after inoculation Three months later the lymph nodes from each mouse were removed and pooled the resulting emulsion being inoculated in a single rabbit, one mouse to one rabbit The rabbits were then observed for the development of darkfield positive lesions Of 45 mice so treated, 44 were shown to be failures as evidenced by the production of darkfield positive lesions in the sub inoculated rabbits With penicillin X the treatment schedule varied from 4,000 to 128 000 units per kilogram of body weight Two such experiments were conducted In the first there were 44 failures out of 46 mice In the second there were 33 failures out of 37 mice These failures occurred at all dosage levels including the highest In a subsequent experiment, two mice were treated with 2 4 million units of penicillin X All of these mice were treatment successes indicating that the CD_{50} for penicillin X in the mouse is some where between 128,000 units and 2 400 units per kilogram Current experiments are being pursued with penicillin X, penicillin G and sodium penicillin, but the results are not yet available

PAROXYSMAL HYPERTENSION ASSOCIATED WITH TABES DORSALIS REPORT OF THREE CASES

By

I L Bennett, Jr, and *Albert Heyman*
(Grady Memorial Hospital Emory University School of Medicine)

This paper describes the clinical and laboratory findings in three patients who developed paroxysmal hypertension asso ciated with tabes dorsalis Although this syndrome was de scribed in detail by Barker and others in 1910 it has received little attention in recent medical literature One of our patients, and another reported from the Mayo Clinic were subjected to unnecessary surgical exploration of the adrenals for suspected pleochromocytoma The exact mechanism of the production of paroxysmal hypertension in these cases is not completely under stood but it is apparently another of the disturbances of the autonomic nervous system that occurs in this disease along with postural hypotension and tabetic bladder Its recognition is important not only in the management of tabes dorsalis but also in avoiding needless surgical exploration of the adrenal glands

THE THERAPEUTIC EFFICACY OF PENICILLIN G IN EXPERIMENTAL SYPHILIS PRODUCED BY FIVE DIFFERENT STRAINS OF TREPONEMA PALLIDUM

By

Ruth A Boak and *Charles M Carpenter*
(Department of Bacteriology and Public Health University of California in Los Angeles)

A comparison of the therapeutic efficacy of penicillin G has been made on five different strains of *Treponema pallidum* to determine whether any variations in their resistance to the drug can be detected Two of these strains have been carried in rabbits for many years the others have been isolated more recently The results suggest that the strains maintained longest in rabbits are somewhat more resistant to treatment with penicil lin than the strains more recently established in rabbits

PENICILLIN TREATMENT OF NEUROSYPHILIS

By
J Lamar Callaway, Sidney Olansky, Arthur H Flower, Jr and *Victor R Hirschman*, Department of Dermatology and Syphilology, Duke University Hospital

Two hundred sixty-nine patients with syphilis of the central nervous system have been treated with penicillin and followed for a minimum of one year Ninety-six patients had asymptomatic neurosyphilis 54 achieved good results, 14 were unimproved, and 28 were lost from observation Seventy-seven patients had general paresis, 40 obtained good results, 19 were unimproved and 18 were lost from observation Twenty five patients had general paresis 40 obtained good results, 19 were patients had tabo-paresis 1 obtained good results, 5 were unimproved and 9 were lost from observation Thirty-two patients were seen with tabes dorsalis of whom 13 showed satisfactory improvement, 6 were unimproved and 13 were lost from observation

PENICILLIN IN CARDIOVASCULAR SYPHILIS

By
Joseph Edeiken and *Mortimer S Falk*
(Penicillin Panel, the University of Pennsylvania)

Fifty patients with cardiovascular syphilis were treated with sodium penicillin in aqueous solution in total dosages ranging from 1 2 to 9 6 million Oxford units Only 4 patients in the series had received metal chemotherapy within a 3 months period prior to penicillin There were 23 with proven uncomplicated aortitis, 20 with aortic insufficiency, 5 with aortic aneurysms and 2 with both aneurysms and aortic insufficiency

Seven patients experienced transient febrile reactions (99 6-102 4°) There appeared to be no relationship between the amount of penicillin in the initial dose and the occurrence of a febrile Herxheimer

Pre-penicillin studies on all patients included thorough physical examinations orthodiagrams, and electrocardiograms More than half of the group (28 patients) also had electrocardiograms at approximately 3-day intervals during the course of treatment. Electrocardiographic changes were noted during treatment in

eight patients—no over all conclusions are warranted but the findings are given in detail in the body of the paper

In no instance were the penicillin injections interrupted or discontinued because of untoward reactions One patient, a 69 year old male with aortic insufficiency and aortic aneurysms, experienced a recurring attack of cardiac asthma on the third day of penicillin therapy The dose per injection was reduced from 40,000 to 10,000 Oxford units for the next 48 hours, and we were able to complete the prescribed course of treatment without further untoward reactions

Five patients with aortic regurgitation and one patient with aortic regurgitation and aneurysm were in congestive failure on admission Penicillin was given along with the measures for counteracting decompensation Treatment was well tolerated and the patients left the hospital improved

Therapeutic paradox, the phenomenon most feared in the treatment of cardiovascular syphilis, did not occur in any of our cases

It is too early to determine whether penicillin will have a beneficial effect in cardiovascular syphilis However, an attempt is being made to follow these patients in the Outpatient Clinic

THE FATE OF WASSERMANN ANTIBODY PASSIVELY TRANSFERRED BY INTRAVENOUS INJECTION OF PLASMA INTO HUMAN SUBJECTS

By

Thomas W Farmer and *Mark M Ravitch*
(Venereal Disease Division, The
Johns Hopkins Hospital)

Each of 16 patients with negative serologic tests for syphilis was transfused with 500 ml of pooled non infectious plasma, obtained from donors with positive serologic tests for syphilis [illegible]

[illegible]
within 20 days after injection Since this serologic response is only transitory, it does not represent a valid objection to the use of plasma with a positive serologic test for syphilis in the blood bank plasma program

THE TREATMENT OF ASYMPTOMATIC NEUROSYPHILIS IN THE WHITE MOUSE

By
Harold J Magnuson and *Barbara J Rosenau*
(The University of North Carolina School of Public Health)

Tryparsamide, mapharsen and penicillin have been assayed against asymptomatic neurosyphilis in the white mouse The CD_{50} of tryparsamide and mapharsen are in excess of the maximum tolerated dose, whereas penicillin (as crystalline G in POB) given daily for four days has a CD_{50} of approximately 32,000 units per kilogram

CARDIOVASCULAR HERXHEIMER REACTION OR COINCIDENCE?

By
R W Maxwell
(Washington University Clinics)

This is a case report of a 44 year old white man who was admitted to the hospital semicomatose, suffering from septic (probably meningococcal) meningitis Immediately after entry penicillin therapy was instituted with individual doses of 50 000 units of aqueous penicillin administered intramuscularly every three hours On the second hospital day his STS was reported as positive and additional history then obtainable indicated that he had untreated syphilis of about 15 years duration At the end of 48 hours, a total of 700 000 units of penicillin had been administered and the patient's temperature was returning to normal At that time a sudden paroxysm of coughing occurred followed by profuse hematemesis and death

At autopsy a severe degree of syphilitic aortitis involved almost the entire aorta Rupture into the esophagus had taken place through a plaque of syphilitic aortitis at the aortic arch.

ACUTE SYPHILITIC MENINGITIS A COMPARISON OF PENICILLIN WITH ARSENO BISMUTH THERAPY

By

Oswaldo A Pardo and *Thomas W Farmer*

(Venereal Disease Division, The Johns Hopkins Hospital)

In the present investigation, the treatment of acute syphilitic meningitis with penicillin is compared with other methods of therapy

Eighteen cases of acute syphilitic meningitis have been treated with penicillin in doses ranging from 600 000 to 10,000,000 Oxford units for periods of 7 to 25 days The period of post treatment observation was from one to four years In 5 of the 18 cases the course was unsatisfactory, and the patients were retreated Among the five failures were two patients with neurorecurrence and three with dark field positive muco cutaneous relapses

Twenty seven patients with acute syphilitic meningitis treated with arseno-bismuth therapy and followed for periods greater than one year after treatment were available for comparison Four of the patients also received inoculation malaria as a part of their first course of therapy The period of follow up observation varied from one to nineteen years In 9 of the 27 cases the results of treatment were not completely satisfactory One patient developed recurrent syphilitic meningitis 4 months after treatment was started Five of the cases had persistent cerebrospinal fluid abnormalities after large amount of treatment, two of these five patients also had persistently positive serologic tests for syphilis Three patients had persistently positive serologic tests for syphilis with normal findings in the cerebrospinal fluid

THE PRESERVATION OF HUMAN PLASMODIA BY FREEZING

By

George M Saunders and *Virgil Scott*
(Washington University Clinics)

The purpose of this paper is to report the successful preservation of human malaria parasites by low temperature freezing Although the preservation of both avian and monkey plasmodia had been described, there had been no report of success with human plasmodia until our preliminary paper in Science, *106* 300, (Sept 26, 1947)

As of the present writing, 14 patients have been successfully inoculated with blood containing human plasmodia preserved for periods ranging from 3 to 129 days by rapid freezing at temperatures of -50° to -70° C Successful preservation has been obtained with each of two strains of *P vivax*, and, although the freezing process results in almost complete hemolysis of the blood and considerable alteration in staining morphology of the parasites, no serious immediate reactions occurred in the recipients and the incubation periods in these patients were not unduly prolonged (10 to 22 days)

The results to date indicate that low-temperature freezing is a practical method of long term preservation of human plasmodia both for use in therapeutic malaria and for preserving strains for experimental purposes

A COMPARISON OF TREATMENT RESULTS UTILIZING CRYSTALLINE PENICILLIN G IN THE TREATMENT OF PATIENTS WITH EARLY SYPHILIS INJECTIONS EVERY TWO HOURS VERSUS INJECTIONS EVERY THREE HOURS

By
Arthur G Schoch (Dallas Syphilis and Venereal Disease Clinic and the Southwestern Medical Foundation)

A series of almost 500 patients with early infectious syphilis was divided into two groups, one in which injections of penicillin were given intramuscularly every two hours, the other group treated in similar fashion every three hours The over-all period of treatment was the same for both groups, namely, seven and one-half days The most significant observation is that the retreatment rate among those treated every three hours was four times as high as among those treated every two hours

PROCAINE PENICILLIN IN SYPHILIS

By
Virgil Scott
(Washington University Clinic)

A study of procaine penicillin (Duracillin, Lilly) has been in progress since early December of 1947 As of Feb 1, 1948, 40 patients with various types of active syphilis, both early and late, have been started on treatment using this material alone It is our preliminary impression that it is highly effective therapeutically and nontoxic

Two regimens have been used—1) 600,000 units semi weekly for 7½ weeks, a total dosage of 9 0 million units, 2) 600,000 units semi-weekly for 10 weeks, a total dosage of 12 0 million units

Therapeutic efficacy—Of the total treated, 12 patients had darkfield positive early syphilis All were darkfield negative on their second clinic visit, three days after their initial injection Earlier darkfield follow-up has been obtained on 4 patients after initial injections of 300,000 units, all of whom were darkfield negative at the time of their first post-treatment darkfield examination (18 to 24 hours).

With three exceptions, all patients have been treated on an

ambulatory basis The 3 hospitalized patients included one each of paresis, primary optic atrophy, and gumma of the liver All of these had febrile Herxheimer reactions after initial injections of 300,000 units In the patient with late hepatic syphilis the temperature rose to 40° C seven hours after the institution of treatment

Toxicity—Pre treatment testing for procaine hypersensitivity has been routinely performed For this purpose 2 per cent procaine solution has been used, 0 1 cc being injected intradermally and 0 5 cc (10 mgms) intramuscularly Although three patients showed dermal sensitivity to procaine, none exhibited any generalized reaction following intramuscular testing The development of procaine hypersensitivity during the course of treatment has not been observed In one patient, generalized urticaria appeared after the fourth injection of procaine penicillin When tested, she was shown to be sensitive to crystalline penicillin G but not to procaine

LYMPHOGRANULOMA VENEREUM OF SUPRACLAVICULAR LYMPH NODES, WITH MEDIASTINAL LYMPHADENOPATHY AND PERICARDITIS, PROVED BY ISOLATION OF THE VIRUS

By

Walter H Sheldon, Margaret Wall, John DeR Slade, and *Albert Heyman*

(Grady Memorial Hospital, Emory University School of Medicine)

This is a report of a young Negro patient who was admitted because of a pericardial friction rub, and roentgenographic demonstration of mediastinal lymphadenopathy The only other significant physical finding was a single large supraclavicular lymph node Histologic examination of this node revealed pathologic changes typical of active lymphogranuloma venereum Hyperglobulinemia, a positive Frei test, and a significantly high titer of complement fixing antibodies for lymphogranuloma venerum were demonstrated Another supraclavicular lymph node appeared several weeks later Histologic examination of this also showed active lymphogranuloma venereum A virus

was isolated from this lymph node by intracerebral inoculation into mice and was identified as the agent of lymphogranuloma venereum It seems reasonable to assume that the mediastinal lymphadenopathy and the pericarditis were also caused by this agent Thorough studies revealed no evidence of other etiology

A COMPARISON OF THE CUMULATIVE FAILURE RATES OF SEVEN DIFFERENT SCHEDULES OF RAPID TREATMENT OF EARLY SYPHILIS WITH PENICILLIN ALONE OR PENICILLIN COMBINED WITH OTHER DRUGS

By
Evan W Thomas

At Bellevue Hospital the cumulative failure rates of seven different schedules of penicillin therapy alone, penicillin com bined with arsenoxide, and penicillin combined with arsenoxide and bismuth are so similar that we must assume that all were about equally effective To analyze the results of the various schedules I have divided the patients in each series into four diagnostic groups (1) seronegative primary syphilis, (2) seropositive primary syphilis, (3) secondary syphilis, and (4) retreatments of infectious relapses or reinfections

Table I gives the statistical results of the seven different schedules of therapy for each of these groups Obviously the four diagnostic groups in each series are not comparable in the num- [illegible] impor- [illegible] in the [illegible] er cent of the so-called failures are actually reinfections, we should have a comparable number of reinfections in each of the four groups If, on the other hand, most of the so-called failures are actually relapses, we would expect a much higher percentage of failures in the secondary groups than in the seronegative and seropositive primary groups Actually there is a difference between the cumulative failure rates of most of the seronegative primary groups and those of the secondary groups, but the differences between the failure rates of the seropositive primary and sec ondary groups are not significant. With most of the treatment schedules, the highest failure rates are found in the groups treated for secondary syphilis but in one of the penicillin G series the seropositive primary group has a higher failure rate

TABLE 1

COMPARISON OF THE CUMULATIVE FAILURE RATES OF SEVEN DIFFERENT SCHEDULES OF RAPID TREATMENT OF EARLY SYPHILIS WITH PENICILLIN ALONE OR PENICILLIN COMBINED WITH OTHER DRUGS

Follow up Through December 1947

	Months of Follow up	Seronegative Primary			Seropositive Primary			Secondary			Retreatments of Infectious Relapses or Reinfections		
		Number Treated	Number of Observed Failures	Cumulative Failure Rate	Number Treated	Number of Observed Failures	Cumulative Failure Rate	Number Treated	Number of Observed Failure	Cumulative Failure Rate	Number Treated	Number of Observed Failure	Cumulative Failure Rate
1146 Patients Treated with 1 200 000 U Penicillin, (20 000 u q 3 hrs for 60 doses) and 0 32 gms Mapharsen (Nov 1944 to Dec 1945)	36	91	15	20 9	214	38	26 9	743	153	33 1	93	21	38 4
807 Patients Treated with 600 000 U Penicillin in Beeswax and Oil Daily for 8 Days (Aug., 1945 to July 1946)	24	62	5	9 0	182	25	18 3	455	72	25 0	108	15	19 2

TABLE 1 (continued)

236 Patients Treated with 2 400 000 U Penic llin (40 000 u q 3 hrs for 60 doses), 0 32 gms Ma pharsen and 5 B smuths (March, 1946 to July 1946)	21	20	1	1 3	64	5	9 1	137	21	21 4	15	0	0
256 Patients Treated with 2 400 000 U Penicillin G (40 000 u q 3 hrs for 60 doses) (July 1946 to June, 1947)	17	32	1	3 2	47	4	9 9	167	18	15 7	10	0	0
251 Patients Treated w th 2 400 000 U Penicill n G (26 666 u q 2 l rs for 90 doses) (July 1946 to June 1947)	17	23	3	18 4	60	6	14 7	154	14	18 3	14	1	25 0
223 Patients Treated with 4 800 000 U Penicill n G (80 000 u q 3 hrs for 60 doses) (July, 1946 to June, 1947)	17	14	0	0	26	4	22 3	97	12	18 9	86	12	18 8
228 Patients Treated with 4 800 000 U Penicillin G (53 333 u q 2 hrs for 90 doses) (July, 1946 to June, 1947)	17	14	0	0	37	4	11 4	95	8	12 3	82	8	13 4

than any of the other groups in the series As a matter of fact among the patients treated with penicillin G the failure rates for the seropositive primary and secondary groups are almost identical

This observed fact can be interpreted in one of two ways (1) There is no difference between seropositive primary syphilis and secondary syphilis with respect to relapses after treatment or (2) the incidence of reinfections is approximately the same following treatment in both groups

I think the second interpretation is probably nearer the truth than the first A careful review of the histories and clinical findings of the relapses or reinfections observed at Bellevue Hospital has convinced me that over 50 per cent were probable reinfections From a statistical basis the only significant factor favoring relapses over reinfections is the fact that the majority of infectious relapses or reinfections have occurred within the first six months after treatment Possibly this observation can be explained by the fact that patients are reinfected by contacts whom they had infected prior to their original treatment, but the fact remains that many fewer relapses or reinfections are observed after six months of follow up than during the first six months

The steady rise in cumulative failure rates with each year of follow-up can be explained by the decreasing number of patients kept under observation and the occasional reinfections which have occurred more than two years after treatment I think it improbable that early syphilis relapses two or more years after treatment and, in my opinion, all of the infectious cases observed more than two years after rapid treatment of early syphilis at Bellevue Hospital were reinfections The cumulative failure rates for the series treated with 1,200,000 units of penicillin and 0 32 gm arsenoxide are based on a follow-up period of at least two years for all observed patients and for three years after that particular schedule of therapy was started For a 17 months follow-up, which would be comparable to that of the penicillin G series, the cumulative failure rates of the 1,200,000 units of penicillin and 0 32 gm arsenoxide series are as follows: Seronegative primary syphilis 14.6 per cent, seropositive primary syphilis—20 4 per cent, secondary syphilis—24 4 per cent, and retreatments—18 8 per cent These results are not as good as those of the penicillin G series but statistically the differences are not significant

AN EVALUATION OF PROCAINE PENICILLIN IN HUMAN GONORRHEA

By
Harold A Tucker, Mark T Hoekenga, Minerva S Buerk, Oswaldo Pardo and *Irving Fien*
(Venereal Disease Division The Johns Hopkins Hospital)

On the basis of studies in over thirty patients with proved gonorrhea, procaine penicillin appears to be more effective than penicillin X or G in aqueous solution

EVALUATION OF ROENTGENOGRAPHIC EXAMINATION IN THE DIAGNOSIS OF INFANTILE CONGENITAL SYPHILIS

By
H Stephen Weens and *Albert Heyman*
(Grady Memorial Hospital Emory University)

Roentgenographic studies of the long bones were performed in a group of 60 infants in whom the diagnosis of syphilis was established Bone changes considered pathognomonic for syphilis were found in approximately 70 to 80 per cent of all patients The criteria for establishing the roentgenographic diagnosis of infantile congenital syphilis are evaluated The incidence of pathognomonic bone lesions varies considerably with the age of the infant The bone lesions in the relapsing cases of congenital syphilis are also discussed In addition roentgenologic bone changes observed in healing are described and evaluated

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SYPHILIS STUDY SECTION*

The Syphilis Study Section of the National Institute of Health is a group of consultants organized for the purpose of promoting and developing research investigations in the field of the venereal diseases The membership of this Study Section is listed below For additional information relative to Syphilis Study Section activities address Frank W Reynolds, M D, Executive Assistant, Syphilis Study Section, National Institute of Health, Bethesda 14, Maryland

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Dr Charles R Rein, New York University

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* The Syphilis Study Section is one of 23 groups of special consultants organized to aid the Division of Research Grants and Fellowships of the [illegible] arch in [illegible] Public [illegible] New [illegible] ite to [illegible] ts and

